Subcutaneous Adipose Tissue-Derived Stem Cells: Advancement and Applications in Regenerative Medicine

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Abbreviations

ADAS	Adipose-derived adult stem cells
AdMSCs	Adipose mesenchymal stem cells
AIT	Autologous islet transplantation
ASCs	Adipose-derived stem cells
AutoHS	Autologous human serum
BAT	Brown adipose tissue
BM	Bone marrow
BMI	Body mass index
BMP	Bone morphogenetic protein
BMSC	Bone marrow stem cell
BSA	Bovine serum albumin
CAL	Cell-assisted lipotransfer
CAM	Cell adhesion molecule
CCl4	Carbon tetrachloride
CD	Cluster of differentiation
CNS	Central nervous system
DEX	Dexamethasone
DMD	Duchenne muscular dystrophy
DMEM-HG	Dulbecco's Modified Eagle's Medium–high glucose
DMEML-G	Dulbecco's Modified Eagle's Medium–low glucose
EBP	Enhancer-binding protein
ECM	Extracellular matrix
FBS	Fetal bovine serum
FGFs	Fibroblast growth factors
GGF	Glial growth factor
GPDH	Glycerol-3-phosphate dehydrogenase
GVHD	Graft-versus-host disease
HA-TCP	Hydroxyapatite/ tricalcium phosphate
HGF	Hepatocyte growth factor
HLA	Human leukocyte antigen
hMADS	Multipotent adipose-derived stem cells
IBMX	3-isobutyl-1-methylxanthine
IFATS	International Fat Applied Technology Society
IFN	Interferon
ISCT	International Society for Cellular Therapy
MEM	Minimal essential media
MSCs	Mesenchymal stem cells
NSCs	Neural stem cells
PDGF	Platelet-derived growth factor
PLA	Processed lipoaspirate
PLGA	Poly(lactic-*co*-glycolic acid)
PPARγ, LPL	Peroxisome proliferator-activated receptor γ, lipoprotein lipase
PTH	Parathyroid hormone
SF	Subcutaneous fat
SLE	Systemic lupus erythematosus
SVF	Stromal vascular fraction
TGF	Transforming growth factor

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TTR	Transthyretin
UCP1	Uncoupling protein 1
VEGF	Vascular endothelial growth factor
vWF	von Willebrand factor
WAT	White adipose tissue

Introduction

Stem cell research has been hailed for its potential to revolutionize the field of regenerative medicine with the ability to regenerate damaged and diseased organs. In addition to offering unprecedented hope in treating many debilitating diseases, stem cells have advanced our understanding of basic biological processes. Intense study on stem cells in the past decade has kindled worthy knowledge about developmental, morphological, and physiological processes that form the basis of tissue and organ formation, maintenance, repair, and regeneration. Today's medicine generally tries to support or treat injured tissues and organs, but stem cells simply replace them [1]. Stem cell research is complicated and rapidly changing. The correlation of stem cell technology with tissue repair still has a long way to go. Since embryonic stem cells are a thorn inside when it comes to the ethics of therapeutics, stem cells isolated from adult tissues sidestep this issue entirely and have become a potent contemporary source of stem cells for tissue repair and regeneration. Conceptually and from a practical standpoint, the bone marrow has been the most influential source of stem cells that offers a possibility of being used in a wide range of therapeutics. Clinical situations frequently demand stem cells with dependable quality and quantity to treat disorders of cellular degeneration. Challenges to bring advances to the clinical mount have expanded rapidly, engendering new perspectives concerning the identity, origin, and full therapeutic potential of various tissue-specific stem cells.

Recent progress in stem cell biology has allowed researchers to investigate distinct stem cell populations in such divergent mammalian tissues and organs such as the tendon [2], periodontal ligament [3], synovial membrane [4], lung [5], liver [6], endometrial tissue [7], and body/tissue fluids such as synovial fluid [8], amniotic fluid [9–11], and menstrual blood [12, 13]. Regardless of the ubiquitous presence of stem cells, taking those stem cells adaptable for regenerative medicine applications in adequate quantities at the right time is a challenge. In this respect, an emerging body of literature suggests that redundant adipose tissue serves as an abundant, accessible, and reliable source of stem cells that can be readily harvested with minimal risk to the patients. Rapidly accumulating evidence suggests that adipose tissue-derived stem cells (ASCs), especially from white adipose tissue, possess a far wider property of self-renewal and multilineage differentiation capacity, thereby highlighting their importance and effectiveness in regenerative medicine [14–18]. Despite literature supporting the plasticity of adipose-derived stem cells for regenerative medicine, there are functional and heterogenous discrepancies associated with it, thus presenting ASC research a difficult and challenging task. Promising strides are continuously being made to unravel these challenges and realize the potential of ASC. While much progress on adipose-derived stem cells has been made in the last few years, there remain a lot to be explored.

This chapter, on the front line, focuses on the overview of current status of knowledge on stem cells derived from white adipose tissue, particularly from subcutaneous fat, representing the salient features of its unique characteristic attributes, viz., isolation and extensive expansion, multilineage differentiation, phenotypic characterization, and media optimization. It also attempts to review the advances that have occurred in recent years in the applications of adipose tissue-derived stem cell technologies in regenerative medicine along with the future promises of the "holy grail" of medicine.

Classification of Adipose Tissue

Adipose tissue is a highly specialized and complex connective tissue that is present in all mammalian species and a variety of nonmammalian species. The primary function of the adipose tissue is limited to the contribution of energy storage in the form of fat. It plays a central role in energy homeostasis, through a network of endocrine, paracrine, and autocrine signals, and has been identified as a highly active metabolic organ [19]. Adipose tissue secretes factors that influence the endocrine regulation of the body, affecting growth, metabolism, and behavior. It exhibits different properties according to its anatomical localization. Functional difference in adipose tissue seems associated with the regional distribution of fat depots, such as white adipose tissue (WAT) and brown adipose tissue (BAT). Despite the difference in functions of both the tissues, they are both named as adipose because of their triglyceride deposits [20].

Brown adipose tissue (BAT), as the name suggests, is brown in color due to the higher cytochrome oxidase content of its mitochondria, which are abundantly found in the cytoplasm. Anatomical distribution of BAT differs from that of WAT. Brown adipose tissue (BAT) is predominantly located in newborns and in hibernating mammals and acts as the primary heat source at a young age. During the natural aging process, brown adipose tissue is gradually replaced by white adipose tissue which is used as a substrate, or starting material, from which energy is generated by a series of biochemical reactions. In contrast, brown adipose tissue, rich in blood vessels and mitochondria, yields energy directly, without intervening chemical breakdown reactions [21]. The cells of brown adipose tissue may be polygonal or ellipsoidal in shape, with a cellular diameter ranging between 15 and 50 μm. BAT contains several smaller lipid vacuoles and a high number of mitochondria that are widely distributed in the cytoplasm and differ in size, giving brown adipocytes a

multilocular appearance [22]. Thus, brown and white adipocytes can be distinguished from each other by size and morphology, abundance of mitochondria, and, most importantly, the presence of uncoupling protein 1 (UCP1) [23]. BAT produces thermogenesis that is spread throughout the body via blood circulation. The regulation of thermogenesis is mainly controlled by the hypothalamus; the sympathetic system carries the signals and releases norepinephrine, which induces fatty acid metabolism, in the mitochondria of brown adipocytes [24]. Heat production is accomplished by the action of a special protein in the inner membrane of the mitochondrion, UCP1, unique to BAT. Brown and white adipose tissues are both innervated by the sympathetic nervous system, which controls metabolic and lipolytic activity as well as the vascularization in adipocytes.

White adipose tissue (WAT) is the only tissue in the body that can markedly change its mass after adult size is reached. In humans, the normal white adipose tissue mass composes 9–18 % in males and 14–28 % in females. In obesity, fat mass exceeds 22 % of the body weight in males and 32 % in females. However, the fat mass can vary depending on the individual ranging from 2 to 3 % of body weight in extremely well-conditioned athletes to even 60–70 % of body weight in massively obese individuals [25]. White adipose tissue is located primarily in three major anatomical areas – subcutaneous (inguinal, dorsal subcutaneous aka axillary and interscapular fat depots), dermal (a relatively continuous sheath of lipids), and intraperitoneal (mesenteric, omental, perirenal, retroperitoneal, epididymal, and parametrial) fat depots. The cells are round or polygonal, ranging between 25 and 200 μm in size. The WAT adipocyte contains a single large lipid droplet in each cell. This droplet does not have a well-defined limiting unit, but has a monolayer membrane between the intracellular lipid component and the cytoplasm [26]. Although these cells contain many organelles, their recognition becomes difficult as the large lipid droplet pushes the organelle, including the nucleus, towards the thin cytoplasm under the plasmalemma. During routine histological processing, lipid becomes dissolved, leaving an empty space that can be seen as a typical signet ring shape under a light microscope [27, 28]. WAT basically functions as (1) energy source for the body along with heat insulation and cushioning, (2) shock absorber on the basis of its anatomical location, (3) has endocrine functions, (4) fills body spaces, and (5) helps lubricate neighboring muscles to allow ease of movement.

Components of White Adipose Tissue

The primary cellular components of white adipose tissue are the so-called adipocytes or lipid-filled fat cells. A population of mature adipocytes contains fat cells of variable size, including very small fat cells (<3.5 μm diameter) [29]. Interestingly, as the population of fat cells increases by diameter or volume with growth or the development of obesity, the heterogeneity of the cell population diminishes. This suggests a maximal adipocyte size that may vary from species to species and from depot to depot. White mature adipocytes consist of about 90 % lipids and each of them stands in close contact with at least one capillary, providing a vascular network that allows continued growth of the organ.

A further integral component of white adipose tissue, apart from the adipocytes, are the stromal cells, identified as stromal vascular fraction (SVF) which is mainly located around the blood vessels. SVF was identified to contain a large population of preadipocytes, first identified by Poznanski et al. in 1971 [30], following enzymatic digestion of adipose tissue. In addition, these harvested adipose stromal vascular fractions were found to possess heterogenous cell population of cells from the microvasculature, such as vascular endothelial cells and their progenitors, pericytes, myeloid dendritic cells, nerve tissue, loose connective tissue matrix, cells present in stroma, and ECM including fibroblast, vascular smooth muscle cells, mesenchymal stem cells, and immune cells such as resident hematopoietic progenitor cells and macrophages [31–33]. Additionally, SVF also possesses leukocytes that may be resident in the parenchyma of adipose tissue [33]. Despite the fact that SVF is a heterogenous cell population, subsequent expansion of the SVF in vitro selects for a homogenous cell population.

Dynamics of White Adipose Tissue

White adipose tissue has a remarkable ability to undergo considerable changes in volume during the life span of an individual. The cellular development associated with relatively small increases in volume can be accommodated by changes in the amount of lipid stored in individual adipocytes (cellular hypertrophy), and larger changes are mediated by the generation of new adipocytes (cellular hyperplasia) accompanied by coordinated expansion and remodeling of the adipose vasculature [26, 34]. Hypertrophy is the result of excess triglyceride accumulation in existing adipocytes due to a positive energy balance. Hyperplasia, or "adipogenesis," involves the recruitment of new adipocytes from precursor cell component in adipose tissue via proliferation and differentiation of preadipocytes. These changes of adipogenesis are mediated first by the proliferation of a population of stem/progenitor cells that are located within the stromal vascular fraction of the adipose tissue to form preadipocytes and second by the process of differentiation, by the form of transition from undifferentiated fibroblast-like preadipocytes into mature round lipid-filled fat cells, characterized by the unilocular appearance of the mature fat cell [35, 36].

These precursor cells are believed to be present throughout the adult life for continuous synthesis of new adipocytes [37]. Thus, from early days, researchers have studied the adipogenic potential of these stem cells using preadipocyte primary

culture (derived from stromal vascular fraction of adipose tissue from various species) and established cell lines of murine origin, committed to adipocyte lineage [38–41], for various reasons: firstly, white adipose tissue is a key player in metabolic homeostasis through its role as both an energy depot and endocrine organ, and secondly, excess adiposity and adipose tissue heterogeneity in obesity are associated with impaired lipid and glucose homeostasis, leading to hyperglycemia, insulin resistance, and type 2 diabetes. Research done so far on the dynamics of WAT, too, indicates that recruitment of lipid-rich preadipocytes from the SVF is done far more rapidly at WAT deposit areas, contributing to the metabolic syndrome. The bulk of the WAT is broadly divided into two main localizations – subcutaneous fat and mesenteric fat, best represented by omentum fat. New insights on WAT biology emerged when the redundant subcutaneous fat, being more abundant and easily accessible, was identified to be the major reservoir of stem cells of potential use for cell-based therapies.

Subcutaneous Adipose Tissue-Derived Stem Cells

Subcutaneous fat is found just below the skin in a region called the hypodermis and intramuscular fat which is found interspersed in skeletal muscle. Like all other fat organs, subcutaneous fat is an active part of the endocrine system, secreting the hormones leptin and resistin. Typical female pattern of body fat distribution around the hips, thighs, and buttocks is subcutaneous fat and therefore poses less of a health risk compared to visceral or the omentum fat. This subcutaneous fat is not related to many of the classic obesity-related pathologies, such as heart disease, cancer, and stroke, and there is even some evidence that it might be protective. Subcutaneous adipose depots are accessible, abundant, and replenishing, thereby providing a potential source as adult stem cell reservoir for each individual. Until the identification of stem cells from omentum fat, the term adipose-derived stem cell (ASC) was the terminology commonly used to refer the stem cells of subcutaneous fat alone. Attention in considering subcutaneous adipose tissue as a reservoir of stem cells was really undertaken only after the findings of Zuk and his co-workers, in the year 2001 [18, 42]. The translation of his findings associated with the easy sampling of adipose tissue with its low risk and morbidity attracted many new investigators. Subsequently, increasing evidence is accumulating on the pivotal role of subcutaneous fat-derived stem cells owing to their proliferative capacity and multilineage differentiation ability [16, 17, 43–47].

In a wide perspective, a range of names has been used to describe the adherent cell population isolated from adipose tissue, e.g., lipoblast, preadipocytes, processed lipoaspirate (PLA) cells, adipose-derived stem/stromal cells (ASCs), adipose-derived adult stem (ADAS) cells, adipose-derived adult stromal cells, human multipotent adipose-derived stem cells (hMADS), and adipose mesenchymal stem cells (AdMSCs) [48, 49]. To address the problem, a consensus reached by investigators at the 2004 conference of the International Fat Applied Technology Society (IFATS) in Pittsburgh has proposed a standard nomenclature by adopting the term ASCs to identify the isolated, plastic-adherent, multipotent cell population [33].

Isolation of Subcutaneous Adipose Stem Cells

In humans, the stromal vascular fraction can be isolated from the redundant subcutaneous adipose tissue, in several regions of the body, especially the abdomen, either by using liposuction aspirate or during reconstructive surgeries like the "tummy tuck" or abdominoplasty. When the starting material is obtained from liposuction procedure, the isolation method is simplified, as the procedure generates finely minced tissue fragments that are more homogenous, allowing a more efficient enzymatic digestion. When working with the solid tissue pieces as starting material, the tissue is minced manually, requiring more time and effort for thorough enzymatic digestion. In 1964, Martin Rodbell was the first to present a method for in vitro isolation of mature adipocytes and adipogenic progenitors from fat tissue obtained from rat [50]. The material is washed after harvesting to remove possible blood contamination. The tissues are minced into small fragments and the extracellular matrix (ECM) holding the adipocytes in place is digested with type I collagenase solution at 37 °C, followed by centrifugation that separates non-buoyant stromal cells from the buoyant adipocytes.

Following this, adipose stem cell research began in 1992 [51], when investigators used cultures of stromal vascular cells isolated from porcine preperitoneal fat in media with heparin and endothelial growth factor, which, they reported, had similar morphology to human subcutaneous adipose cells. This produced cells that stained positive for von Willebrand factor (vWF), α-smooth muscle cell actin, and cytokeratin and were termed as microvascular endothelial cells. Subsequently, the stromal vascular fraction has been shown to include multipotent mesenchymal stem cells, which may reside in the perivascular region of the stroma. Zuk and co-workers were the first to show that the SVF fraction isolated from human liposuction samples contained cells with multilineage potential and termed as processed lipoaspirate (PLA) cells [18, 42]. They allowed the ASCs to adhere to the plastic surface of tissue culture flasks, which is still the basis of most methods used to date. Since then, several groups working independently have developed and refined procedures of isolating and characterizing adipose-derived stem cells obtained from both lipoaspirates and the excised solid fat tissue obtained through lipectomy [18, 35, 43, 53, 54].

The yield of stromal vascular fraction obtained using different harvesting techniques such as solid fat tissue through reconstructive lipectomy surgeries, tumescent or conventional liposuction, and ultrasound-assisted liposuction and harvesting sites, such as subcutaneous fat of the abdomen and hip/thigh, has also been investigated. Fraser and his co-workers contended that neither the site of harvest nor the harvesting technique affected the number of stem cells obtained [52]. On the contrary, Varma and his co-workers suggested that the harvesting technique affected the recovery of ASCs, with ultrasound-assisted liposuction yielding the lowest number of proliferative ASCs [53]. Later, the same group and other researchers also identified that the site of harvest affects the yield of ASCs. They reported that frequency of cells in abdominal subcutaneous fat is much higher than the frequency obtained in hip/thigh subcutaneous fat [43, 53, 54]. In addition, Varma and his co-workers also reported that SVF cells derived from abdominal fat reach 80–90 % confluency within 5 days, whereas SVF cells derived from adipose tissue of the hip/thigh take more than 9 days to reach 80–90 % confluency when seeded in the same density [43]. To conclude, it is probable that subcutaneous fat obtained from the abdomen for SVF isolation is the better harvest site when compared to the hip/thigh.

Yield comparison derived from lipoaspirates as well as from lipectomy identified from the subsequent work suggested that abundant number of ASCs can be derived from lipoaspirates as well as from lipectomy procedure. The non-buoyant stromal cells were identified to yield in excess of $1\times10^6-6\times10^8$ ASCs from stem cells from 100 to 300 cc of abdomen fat in comparison to 2.5×10^4 stem cells from the 40 cc of bone marrow aspiration [14, 18, 45, 54–56], with more than 90 % of the cells being viable. Owing to the greater concentration of cells from subcutaneous fat and its favorable characteristics, it was reported that the ASCs can directly be used in different therapeutic doses for treating a variety of diseases without any further expansion in culture [49, 57–60].

Phenotypic Characterization of Subcutaneous ASCs

Despite nearly a decade of research efforts, the phenotypic and functional characteristics of ASCs remain obscure. Although fibroblastic morphology of cells is one of the characteristics to identify MSC, such characterization alone is insufficient to confirm the isolation of a true ASC population. Thus, immunophenotypic characterization of cell surface markers becomes an ideal characteristic feature of stem cell. It is uncommon to find a protein whose expression is completely specific to one particular cell type and is therefore usually necessary to build an expression profile which considers a range of markers known to be expressed by a given cell type. Many attempts have been made to develop a cell surface antigen profile, in order to better purify and identify ASCs, especially a common immunophenotype which could enable isolation of a purified population of ASC. However, to date, no single marker has been identified that delineates ASC in vivo, and hence, there is a lack of thorough understanding of the mechanism underlying stem cell renewal and its functional differentiation. Thus, quest for the identification of a prospective definitive biomarker remains elusive. The simple way of identification is from the progressive loss of hematopoietic cell lineage markers that can reliably identify ASCs exclusively.

Over the past 6 years, many papers have reported on characterization of cell surface markers at stromal vascular fraction or at different stages of ASC culture. Considerable progress has been made towards phenotypic characterization of ASC; however, the expression profile changes as a function of time in passage and plastic adherence [61, 62]. Besides, some cell surface markers have been detected with highly consistent patterns of expression (CD13, CD29, CD73, CD90, CD105, CD166, CD133, CD45, CD31, MHC I, and MHC II) on the surface of ASCs by different literatures [16, 44, 47, 58, 63–65]. Still considerable heterogeneity, in the full range of ASC surface markers, had been reported. Some of these variations are found in the cell population within a single culture, indicating either the presence of mixed population of cells or the modulation of cell surface proteins during cell culture. Furthermore, the comparative analysis of phenotypic expressions had been confounded by the differences in thresholds used to report positivity in staining by different research groups [42, 49, 66].

To circumvent these barriers, minimal criteria with set of standards for identifying the MSC population from all sources have been proposed by the mesenchymal and tissue stem cell committee of the International Society for Cellular Therapy (ISCT) [67]. They reported that 95 % of the MSC population must express CD105 (known as endoglin and originally recognized by the Mab SH2), CD73 (known as ecto-5-nucleotidase and originally recognized by the Mab SH3 and SH4), and CD90 (also known as Thy-1), as measured by flow cytometry. Additionally, these cells must lack expression of CD45 (a pan-leukocyte marker), CD34 (primitive hematopoietic stem cells and endothelial cells), CD14 or CD11b (a monocyte/macrophage marker), CD79a or CD19 (markers of B cells), and HLA class II (expressed when stimulated by IFN γ). This standard has been accepted and followed for the identification of MSC by the scientific communities around the world.

Despite the in vitro identification of ASCs made possible through these aforesaid markers, their role with respect to other cell populations, such as side population, endothelial progenitor population, and specifically cell adhesion molecules within extracellular matrix, is not clear and there is a lack of consensus in the results. Although ASC shows very similar expression patterns as that of BMSC, it still remains unclear in the aspects of ASC specific marker [16, 44, 47, 58]. On the contrary, there are also reports specifying the variations exhibited among certain markers of ASC when compared to

BMSC and vice versa [14, 42, 47, 54, 63], further making cell surface marker expression study on ASC an arduous task. For example, it was identified that ASCs express CD49d and not CD106, whereas bone marrow MSCs express CD106 but not CD49d. This reciprocal expression pattern is interesting because CD106 is the cognate receptor of CD49d, and both these molecules represent part of a receptor–ligand pair that has an important role in hematopoietic stem cell homing to, and mobilization from, the bone marrow [68–71]. Despite the lack of CD106 in ASC, CD49d along with the higher expression of its counterpart, CD29, together forming VLA 4 is supposed to play a role in mobilization and homing [44, 47, 49, 54, 60, 72, 89]. In addition, it is predicted that high expression of CD44 is required for firm adhesion of MSC to endothelium. Data shows that CD44 is activated by PDGF [73] that plays an important role in exogenous migration to injured site by interaction with hyaluronate. Besides, CD13 was also identified to be a potent marker that plays a vital role in angiogenesis and migration. This wealth of knowledge on these markers about their crucial migration and homing evokes that these markers impersonate a CAM that performs these aforesaid functions in ASC. Although the existence and functionality of certain ASC specific markers are known, there is uncertainty among the specificity and functionality of several other markers of ASC.

Overall, the ASCs were identified by expressions of various markers such as STRO1, CD9, CD10, CD13, CD29, CD44, CD90, CD49a, CD49b, CD49d, CD49e, CD49f, CD54, CD55, CD59, CD166, CD71, CD117, CD19, CD146, and HLA-ABC. Similarly, there is evidence supporting that the surface markers CD31, CD45, HLADR, CD11b, CD14, and CD34 were decreased in expression or were lost with passage, suggesting that adherence to plastic and subsequent expansion will select for a relatively homogenous cell population compared with the SVF [18, 45, 47, 48, 54, 63, 65]. One of the key issues yet to be resolved is the absence of comprehensive information on certain specific markers of ASC, especially the cell adhesion molecule that interacts with the cytoskeleton of MSC. This might enhance the understanding of ASC as an instrument of curative therapeutics involved in the applications of neovascularization, angiogenesis, and treatment of other vascular disorders. Furthermore, these definitive cell surface markers of ASC would help not only to distinguish them from other cell populations in cell culture but also enable purification of ASC from uncultured SVF. As uncultured heterogenous SVF is been used in clinical trials extensively for treating a wide horizon of diseases, it is imperative to identify the heterogenous cell surface markers specific to SVF. The description on the existence of heterogenous and homogenous cell surface markers that can reliably specify ASC as depicted (Table 10.1) facilitates researchers to explore further therapeutic potentials of stem cells with SVF as well as ASC.

Table 10.1 Comprehensive analysis of cell surface markers in SVF and ASC

Markers	SVF	ASC	
Hematopoietic markers	CD34#	+	–
	CD14	ND	–
	CD45	ND	–
	CD117	ND	+
Mesenchymal markers	CD90	+	+
	CD73	+	+
	CD105	–	+
CAM molecules	CD49d#	+	+
	CD49a	+	+
	CD49b#	ND	+
	CD49e	+	+
	CD61#	ND	+
	CD62e#	ND	+
	CD63#	+	+
	CD54#	+	+
	CD50	ND	–
	CD51	ND	+
	CD56	ND	–
	CD55	ND	+
	CD29	+	+
	CD11a	ND	–
	CD11b	ND	–
	CD11c	ND	–
	CD44	+	ND
	CD31	+	–
	CD9	ND	+
	CD106	+	–
	CD166	+	+
	CD104	ND	–
	CD144	–	ND
	CD146#	+	+
Surface enzyme	CD13	+	+
	CD10	ND	+
	ALDH	+	+
Growth factors	CD140b	ND	+
	VEGFR-2	–	–
Histocompatibility antigens	HLA-ABC	ND	+
	HLA-DP	–	–
	HLA-DQ	–	–
	HLA-DR	–	–
Side population	ABGC2	+	+
Cytoskeleton marker	α-SMA, vimentin	ND	+
Complement cascade	CD55	ND	+
	CD59	ND	+
Receptor molecules	CD71	ND	+
Endothelial marker	von Willebrand	–	–
Pluripotent markers	Oct-4	ND	+
	NANOG	ND	+
	SOX2	ND	+

Refs. [14, 42, 44–47, 52–54, 63, 65, 75, 92, 152, 186–193, 208]
+ expressed, – not expressed, # variable, *ND* not done

Differentiation Potency

Another property put forward by ISCT that potentially identifies MSC is by its differentiation potency. The cells must be able to differentiate to mesodermal lineages of osteoblasts and adipocytes to demonstrate bone and fat phenotypes, respectively, under standard in vitro differentiating conditions [67]. To investigate multipotency, several researchers had demonstrated the multilineage differentiation ability of subcutaneous adipose-derived stem cells [15, 16, 42, 74, 75]. For instance, Rodriguez and his co-workers [76] created a single clone from fast-adherent ASCs and proved that 2 out of 12 clones were able to undergo multilineage differentiation [48]. The remaining ten clones had bipotent capacity. These findings indicate that a high percentage of ASCs have multipotential and pluripotential capacity in vitro to differentiate into the major mesodermal and ectodermal lineages. Adipose tissue-derived mesenchymal stem cells naturally differentiate into mature adipocytes [15, 16, 42, 74, 75]. Upon treatment with adipogenic induction medium containing 3-isolbutyl-1- methylxanthine (IBMX), dexamethasone (DEX), indomethacin and insulin, ASCs were found to develop intracellular lipid vacuoles which coalesce and give rise to a single, cytoplasm filling vacuole. Besides, the definite markers of adipogenesis [76], ASCs also express a wide variety of metabolic markers such as glycerol-3- phosphate dehydrogenase (GPDH), lipoprotein lipase, peroxisome proliferator-activated receptor γ(PPARγ), leptin, adipocyte fatty acid-binding protein (Ap2)11, CCAAT/enhancer-binding protein (C/EBP), and glucose transporter 4 (Glut4).

Similarly, ASC differentiation towards the osteogenic cell lineage is well established for in vitro and for in vivo animal tissue engineering models [15, 16, 42, 74, 75, 77]. A clinical observation is in part responsible for the discovery of the osteogenic differentiation capacity of ASCs. A rare disorder named "progressive osseous heteroplasia" together with the capacity of MSCs to convert into the osteogenic lineage led to the assumption that ASCs are likewise able to differentiate into osteocytes [78]. Osteogenic induction of ASCs can be achieved by similar culture conditions as used in MSCs, including supplementation with ascorbic acid together with 1-alpha,25-dihydroxyvitamin D3 (1,25(OH)2D3), the hormonal metabolite of vitamin D, or dexamethasone [79]. Under osteogenic differentiation medium, ASCs are capable of expressing diverse genes and proteins found in the osteoblast's phenotype: type I collagen, alkaline phosphatase, osteocalcin, osteonectin, osteopontin, parathyroid hormone (PTH) receptor, bone morphogenetic protein-2 (BMP-2), BMP-4, BMP receptors I and II, bone sialoprotein, and RunX-1 [18, 79, 80].

Earlier, mesenchymal stem cells were considered as capable of only forming tissues of mesodermal origin, as has been demonstrated above. However, in recent years this phenomenon has been challenged by several reports, and it is no longer surprising that mesenchymal stem cells, having originated in the mesoderm, can cross the germ layer boundary and undergo different mesenchymal lineage conversions of ectodermal and endodermal origins. This process has been termed as transdifferentiation or plasticity. This is evident from the clonal expansion of adipose-derived stem cells which proved that at least one part of the plasticity is situated in a fraction of multipotent cells [45]. This concept that mesenchymal stem cells possess a far wider potential of transdifferentiation is also supported by literatures [81]. The perception of the far wider potential of multidifferentiation potency of ASCs as depicted (Table 10.2) has led to considerable excitement with regard to its potential therapeutic applications in regenerative medicine and tissue engineering [15, 59]. Nevertheless, further needs to be explored on the differentiation potency of ASCs in order to achieve curative therapeutics for all diseases.

Prerequisite for Ex Vivo Expansion

Adipose tissue-derived stem cells were identified to possess the key characteristics of MSCs as reported by ISCT, including the ability to form plastic-adherent fibroblastic morphology, extensive proliferative capacity, the ability to express several common cell surface antigens, and the ability to differentiate into several mesodermal lineages, including the bone, fat, and cartilage. Hence, ASCs are recognized an attractive and abundant stem cell source in recent years, with therapeutic applicability in diverse fields of tissue repair and regeneration. To take this initiative to the next step, on the front line, it is important to isolate and generate a large clinical quantity of MSCs, while retaining stem cell characteristics, immunosuppressive capacity, and multilineage differentiation potential. Reaching the estimated clinical dose of cells per kilogram body weight of adult patients is highly challenging. Hence, in vitro expansion of MSC is a prerequisite to obtain clinical dose for subsequent therapeutic applications using ASCs.

The use of MSCs for clinical approaches in many fields of medicine first requires that the biosafety of these cells be carefully investigated through appropriate and sensitive tests. Indeed, documentation on the absence of transformation potential in cultured MSC before infusion of these cells into patients is of utmost importance. Furthermore, as MSC possesses a greater propensity for ex vivo expansion, ISCT reported that extensively passaged cells may be well served by verifying a normal karyotype to reduce the probability of chromosomal abnormalities, including potentially

Table 10.2 Induction of multilineage differentiation of ASCs in vitro

Germ layer	Induction factors	Genes upregulated
Mesoderm		
Adipocytes	3-Isobutyl-1-methylxanthine (IBMX) + dexamethasone(DEX) + indomethacin + insulin [18, 207]; ascorbic acid + insulin + sodium selenite + triiodothyronine + IBMX + DEX + rosiglitazone [48]; IBMX + DEX + insulin + pantothenate + biotin + rosiglitazone [45]	PPARγ, CCAAT/enhancer-binding protein α (C/EBP α), lipoprotein lipase (LPL), adipocyte fatty acid-binding protein (FABP4/aP2), leptin
Osteoblast	DEX + ascorbate-2-phosphate + β-glycerophosphate [18, 207, 208];1,25$(OH)_2D_3$ + β-glycerophosphate + ascorbic acid + BMP-2 + DEX + valproic acid [77, 194–196, 209, 210]; ascorbate-2-phosphate + β-glycerophosphate + BMP-2 [43]	Type I collagen, alkaline phosphatase, osteocalcin, osteonectin, osteopontin, PTH, BMP-2, BMP-4, BMP receptors I and II, RunX-1, Bone sialoprotein, ALP
Chondrocytes	Insulin + TGF- β + ascorbate-2-phosphate [18, 197–199, 201, 211]; DEX + ascorbate-2-phosphate + sodium pyruvate + TGFβ1 + ITS premix [43], TGFβ3 + BMP-6 + DEX + ascorbate-2-phosphate + proline + pyruvate + ITS premix [208]	COLL II, COLL VI, aggrecan
Cardiomyocytes	5-azacytidine [206] Transferrin, IL-3, IL-6, VEGF	Connexin-43
Myogenesis	Hydrocortisone + DEX [18]	Desmi, MyoD, dystrophin, telethonin
Endoderm		
Beta cells	Glucose + nicotinamide + activin-A2 + exendin-4 + HGF + pentagastrin + B27 + N2 [81]	Isl-1,Pax-4,Pax-6, PDX-1, Ngn-3, insulin, nestin, glucagon, somatostatin, NeuroD, Nkx2.2, Glut2, Ipf1
Hepatocytes	HGF + bFGF + nicotinamide + DEX + insulin + OMS + transferrin + selenious acid + linoleic acid + BSA + ITS [204] DMSO + rhHGF + rhOSM [168]	Albumin, α-fetoprotein, enhancer-binding protein beta C/EBPβ, albumin (ALB), transthyretin (TTR), cytochrome 2E1 (CYP 2E1)
Ectoderm		
Neurons	β-Mercaptoethanol [42]; β-mercaptoethanol + trans-retinoic acid + PDGF + bFGF + GGF-2 [205]; butylated hydroxyanisole + valproic acid + forskolin + hydrocortisone + insulin + KCl [202];	
5-azacytidine + NGF/BDNF/bFGF + B27 [164]	MAP2, γ-enolase, NeuN, intermediate filament m, nestin, glial fibrillary acidic protein (GFAP), β-III tubulin, oligodendrocyte marker O2, glutamate receptor subunit NRN 1 and NRN 2	

transforming events [67]. Considering these facts, there have been continuous attempts in optimizing culture conditions for ASCs that retain stem cell characteristics that could be beneficial for clinical and therapeutic applications [46, 82–84].

Despite the wide prevalence of mesenchymal stem cells from adipose tissue and their observed benefits to their use in both in vitro and in vivo models of certain human diseases [15, 16, 42, 74, 75, 77], clinical trials using ASC have not reached major success. Research exists on overcoming the barriers to maximize the beneficial effects of ASC for their use in treating wide horizon of diseases. One such anticipated barrier might be due to failure of the transplanted cells to survive, proliferate in numbers, and differentiate into tissue-specific cells, which could then incorporate into diseased tissue, repopulate the area with healthy tissue, and thereby exerting an appreciable effect of improvement. Although the reason for the same is not fully understood, it is hypothesized that MSC might enter senescence and start losing their characteristics soon after infusion [85]. Hence, optimization of culture condition and maintenance of ASC characteristics under extensive culturing in vitro are crucial to resolve this issue.

Furthermore, it was identified that ASCs have profound immunomodulatory effects in vivo and are regarded as hypoimmunogenic cells [65], and hence ASCs from allogenic donors might constitute a valuable alternative source of stem cells for their use in case of transplantation. Thus, ASC can be a promising tool that forms the basis for new strategies in immunoregulatory cell therapy and allogenic cell transplantation in regenerative medicine. In order to achieve this, ASCs have to be expanded extensively to yield cells that retain its characteristics substantially enough to give appreciable effect to cure diseases. By achieving this attribute, a large quantity of allogenic donor-derived MSCs can be obtained from subcutaneous adipose tissue in a less time-

consuming, noninvasive, cost-effective, efficient, and safe source of clinical cell transplantation.

In accordance to these, several reports have focused on studying the efficacy of retention capacity of its characteristics at prolonged culture condition of both rat and human adipose tissue-derived mesenchymal stem cells. Both murine and human stem cells were found to retain their properties evidence for the possibility of their characteristics until prolonged culturing. They were able to preserve their long-term stem cell characteristics and differentiation potential even at longer passages [46, 82, 83]. The human subcutaneous adipose tissue showed high telomerase activity that could be maintained for more than 100 population doublings. The karyotype was found to be normal and it was positive for certain pluripotent markers retaining its efficacy until later passages. Thus, evidence is available proving that subcutaneous adipose tissues possess properties of true stem cells, which were retained even after extended in vitro culturing, thereby rewarding a prerequisite for possible successful cell-based therapies. However, on the contrary, ASC under extensive expansion condition was reported to lose its characteristics of retention and differentiation ability [84, 86, 87]. In addition, another group observed spontaneous malignant transformation of adipose-derived hMSC in vitro [88], thus creating a perplexity and hindering the progress of ASC towards therapeutic interventions.

Hence, enhanced research work on ASC regarding maintenance of its quintessential properties in early and later passages without genetic or epigenetic changes before its use in clinical transplantation is of utmost importance. In accordance to this, our laboratory had reported recently that ASCs in early passage condition obtained from various donors (difference in age, gender, and BMI) showed a consistency related to its expression of cell surface markers, growth potency, and differentiation ability [89]. This study, along with the reports of immunomodulatory properties of ASCs explained earlier [65], thus, demonstrate the importance of cryopreservation of cultured ASCs. This identification puts forth a breakthrough challenge of using these cultured ASCs towards clinical applications that can be subsequently used for effective tissue repair and regeneration in a noninvasive manner. However, in order to achieve this challenge, our group investigated whether subcutaneous adipose-derived mesenchymal stem cell could be retained in extensive culturing without losing their property. The hypothesis was achieved by culturing the ASC in five different media, viz., DMEM LG, DMEM HG, Alpha MEM, DMEM F 12, and DMEM KO, as media used for cell culture have a significant impact on growth and differentiation of ASC. We proceeded a step forward to demystify a number of myths of ASC. In our study, optimization of culture media was successfully achieved by extensively propagating mesenchymal stem cells from subcutaneous fat until passage 25. The study revealed the possible use of ASCs from subcutaneous fat in curative therapeutics by demonstrating retention characteristics of MSC for more than 6 months in all media used, which was confirmed by various attributes in view of its expression profile, proliferation, differentiation, and normal karyotype in its extensive culturing condition. Furthermore, *optimization of basal media appropriate for culturing ASCs from subcutaneous fat based on its growth curve and population doubling time was also identified, thereby permitting the scientist to choose their pertinent media according to their type of research and therapeutic implications* [90].

Yet another obstacle that can potentially hinder the application of cultured ASCs for its use in cell-based therapies is the fetal bovine serum (FBS), as culturing cells aimed for clinical therapy in FBS is an unsuitable option with respect to patient safety [91]. Although from cell culture point of view FBS provides the cells with essential nutrients and growth factors [92], species of origin and serum concentrations affect the proliferation of ASCs [93–95]. Human cells exposed to xenogeneic (i.e., animal derived) products originating from cell culture reagents may transfer xenogeneic antibodies, such as Neu5GC, into the human body upon transplantation, raising the risk of triggering a severe immune response in the recipient [96–98]. Several researchers have thus focused on identification of FBS replacement with human serum derivatives to support equal or higher proliferation rates and multilineage differentiation capacity of ASCs [93, 95, 99, 100]. AutoHS (autologous human serum) is perhaps the obvious option for clinical applications, since it eliminates the problem of introducing xenogeneic or allogenic antibodies into the patient. However, there are conflicting results of the superiority of autologous human serum compared to FBS in terms of proliferation rate and differentiation potential [101]. Oreffo et al. [102] reported improved osteogenic and adipogenic differentiation using autoHS compared to FBS, and Yamamoto et al. [103] showed similar results for osteogenic differentiation using autoHS versus FBS. However, utilizing autoHS for large-scale stem cell production for clinical applications is impeded by limited availability and high variability in cell growth in autoHS [92, 101, 104, 105]. Development of completely defined, SF/xeno-free (XF) medium compositions for expansion of adult stem cells is still in its infancy with only few papers published, however, with highly promising results [106–108]. Importantly, the culturing formula must also be capable of expanding the cells multifold in a minimum number of passages, since long-term in vitro culture may be time consuming and at times might alter the biology of ASC [109, 110]. Further research pursuit in this aspect might create a hope in the light of existing perplexity to bring subcutaneous fat as a frontline source of both autologous and allogenic therapeutic stem cells in the field of regenerative medicine.

Applications in Regenerative Medicine

Current treatments for various degenerative disorders rely on surgical interventions and drugs that modulate the system, but these have their own limitations when it comes to regeneration of damaged tissues and cells. To address this shortcoming, cell-based therapies are gaining importance. Regenerative medicine is a multidisciplinary field of research that is rapidly expanding with the advances in the technologies. It involves the use of a stem cell, growth factor, and a biomaterial that restores function by enabling the body to repair, replace, and regenerate or rejuvenate damaged, aging, or diseased cells, tissues, and organs. Adult stem cell research has been hailed for its potential to revolutionize the future of regenerative medicine with the ability to regenerate damaged and diseased organs. Detailed study on adult stem cells in the past decade has kindled useful knowledge about developmental, morphological, and physiological processes that form the basis of tissue and organ formation, maintenance, repair, and regeneration.

Conceptually, and from a practical standpoint, the bone marrow has always been considered a rich source of stem cells for regenerative medicine. It is known to possess heterogenous stem cells that participate in all steps of hematopoiesis and tissue regeneration. Despite its colossal potential and benefits in certain diseases, stem cells derived from the bone marrow have not been promising to attempt curative therapeutics for all diseases. Hence, adipose tissue-derived stem cells flourished as an alternative source to play a potential pivotal role in regenerative therapeutics. Subcutaneous adipose tissue is an abundant, accessible, and replenishable source of both uncultured/heterogenous stromal vascular fraction cells and cultured/relatively homogenous adipose-derived stem cells. The basic, experimental, and clinical research on SVF/ASC has expanded exponentially over the past decade. Cell-based therapy using ASCs presents a unique opportunity for their use in tissue repair and regeneration. The important experimental findings using SVF/ASC in recent years in treating wide range of diseases are detailed below, thereby laying a blueprint for ASC in cellular replacement and regenerative medicine.

Mesodermal Applications

Bone Diseases

Bone diseases, such as osteopenia and osteoporosis, affect several millions of patients throughout the world. Ongoing studies for repairing bone defects are performed in pharmacology, gene, and cell therapies. Suitable cells needed for tissue engineering should exhibit immunocompatibility and self-regenerative potential. Osteoblast differentiation represents a crucial event during skeletal tissue formation, bone repair, and bone remodeling [111]. The in vitro osteogenic potential of ASCs has been documented in multiple studies [18, 42, 74, 79, 80, 112, 113] as described above. Furthermore, these cells have also proven to regenerate bone defects in the in vivo animal and human models as elucidated below. In efforts to utilize this potential for tissue-engineered bone repairs, many laboratories have begun seeding osteogenically differentiated ASCs onto various scaffolds and biomaterials. The biomaterials that rendered the most significant result are PGA [74], atelocollagen [112], and hydroxyapatite/tricalcium phosphate (HA-TCP) [114]. The first use of autologous ASC for osseous repair has been reported in the treatment of craniofacial defects, for a bilateral calvarial defect in a 7-year-old girl [115]. Due to the restricted amount of BM available in patients with calvarial defects, the trial followed a complex clinical course characterized by the application of ASC to the defect in a single procedure together with autologous iliac crest bone and fibrin glue combined with resorbable sheets. New bone formation as well as nearly complete calvarial continuity was evident 3 months after the reconstruction. It is believed that strong paracrine signaling from the underlying dura mater and the osteoinductive properties of the apatite coating may have played a role in these divergent outcomes. Further successes have been observed in a rat cleft palate model. Osteogenically differentiated ASCs seeded onto polylactic acid scaffolds led to near-complete palatal repair at 12 weeks when implanted into the defect. In comparison, no bone formation was seen with acellular scaffolds and those seeded with undifferentiated cells [116]. Furthermore, the author harvested autologous fat tissue from a 65-year-old male who had undergone a hemimaxillectomy 28 months earlier due to a large recurrent keratocyst, expanded the cells in culture, mixed them with BMP-2, and seeded them onto a beta-tricalcium phosphate scaffold formed into the shape of the defect. Eight months after this construct was implanted into the patient's rectus abdominis muscle, the construct was resected and transplanted into the maxillofacial defect. The patient regained full oral function. Although evidence to date suggests that ASCs may one day be useful in the treatment of difficult osseous repairs, further investigations are needed to determine their ultimate safety and efficacy in the clinic.

Soft Tissue Defects

One of the most intuitive uses of ASCs is for the replacement of adipose tissue itself. Large soft tissue defects are a common problem following trauma, burns, and oncological resections, such as mastectomy. In addition, thousands of patients annually require cosmetic treatments to smooth wrinkles, fill in cheeks, and otherwise augment natural subcutaneous adipose compartments. The material currently used in soft tissue regeneration, which includes collagen, hyaluronic acid,

silicon, and other filler materials, has several disadvantages such as high cost, immunogenicity, and allergenicity and the risk of transmitting infectious diseases. Although autologous fat grafts are available, their one limitation is the poor long-term graft retention in current clinical practice [117]. The transplanted graft can lose volume over time due to tissue resorption that can result in the loss of the original graft volume [118]. In order to develop more physiological alternatives for soft tissue reconstruction, several laboratories have investigated the possibility of creating tissue-engineered cell-seeded scaffolds for the generation of de novo adipose tissue. The ideal solution for soft tissue regeneration would promote the regeneration of vascularized adipose tissue to completely fill the defect volume. Min et al. demonstrated in an in vivo murine model that the transplantation of fat tissue with non-cultured ASC improved long-term graft retention and a higher density of capillaries 6 and 9 months after transplantation [117]. The reasons for these successful results might be the proangiogenic growth factors secreted by ASCs. Initial success rate has been seen in animal models injected with cells seeded with artificial scaffold [119–121]. In each trial, cell-seeded grafts showed significant neovascularization of the implant, as well as penetration of the preadipocytes or ASCs into the scaffolding, and their differentiation into mature lipid-laden adipocytes. However, the influence of variables such as porosity, biomaterial composition, and seeding density has been under continuous investigations for the optimization of the constructs to improve adipogenesis.

Yoshimura and co-workers used adipose-derived SVF cells for soft tissue augmentation by a novel strategy called cell-assisted lipotransfer (CAL) for treatment of facial lipoatrophy and for breast augmentation. It was identified that ASC supplementation has improved its efficacy and improved facial contour, with no adverse effects, although there is no statistically significant difference identified. Furthermore, breast tissue augmentation and reconstruction trial had been reported by Yoshimura and colleague [122, 123]. The SVF was isolated from half of the aspirated fat tissue, recombined with the remaining half, and used in combination with lipoinjection in over 50 patients. These results showed no evidence of fibrosis or adhesions and improved fat grafting by the SVF cells with the retention of volume for 12 months. Developing strategies to reconstruct larger tissue defects, however, remains a formidable challenge due to the inherent ischemic conditions within larger transplants and the time necessary for the establishment of an extensive vascular network.

Cartilage Defects

Clinical cartilage repair has remained an elusive goal for some time. Autologous [124] and allogenic [125] chondrocyte transplants have been used successfully, but are limited by donor site morbidity and the slow repairs seen, respectively, with these approaches. Recognition of the chondrogenic differentiation potential seen in many stem cells has led to the exploration of an alternative source of cells. Chondrogenic potential, described in vitro in ASCs, includes evidence of cell condensation into nodules and the production of an extracellular matrix rich in proteoglycans and collagen type II [126–131]. It has been proved successful for a minimum period of 12 weeks when implanted with alginate constructs subcutaneously in nude mice [129]. A direct comparison of the in vitro chondrogenic potential of ASCs and BMSCs examined similarities in histological staining and gene expression [131]. In vivo experiments using ASC spheroids had been identified and were successful at generating cartilage-like tissue [132]. Induced spheroids were implanted between two muscle bellies in immunodeficient mice. At 6 weeks, the implants were harvested and found to have produced a cartilage-like tissue consisting of cells within lacunae surrounded by a gel-like extracellular matrix in the absence of any fibrous network. Although this study demonstrates an in vivo potential for differentiation intramuscularly, no physiological models of cartilage repair have yet been tested. The cartilage, particularly articular, primarily serves a structural and mechanical function in the body. To this end, one laboratory is investigating what effects scaffold material [133, 134], oxygen tension [133], and media composition [133] have on the biomechanical properties of ASC-seeded constructs. Clear differences are seen depending on the combination of factors used; however, nothing yet approaches the mechanical properties of mature cartilage. Thus far, results suggest that future in vivo models may demonstrate a potential for ASCs to enhance the healing of debilitating osteochondral diseases. Repairs with the resilience necessary for weight-bearing joints, however, will probably be more difficult to develop. It remains controversial whether ASC cells are perfect for cartilage engineering. However, ASC seems to have a greater chondropotential effect and further enhanced work on the same might improve engineering of cartilage in vitro as well in vivo.

Cardiac Repair and Neovascularization

Congestive heart failure, a common clinical condition that results from acute ischemic events and diffuse progressive weakness, is essentially a failure of the myocardium. Existing pharmaceutical drugs in the market can temporarily resolve the effect, but later drive the heart harder and might further weaken the muscle and play a little role to repair damaged tissue. The use of stem cells to regenerate damaged heart tissue is advocated as the new treatment for heart failure secondary to heart disease or severe myocardial infarction. Several recent reports suggest that ASCs may emerge as a

promising source for cardiac therapeutics. In vitro differentiation, including morphological changes, spontaneously beating foci, and the expression of cardiac-specific markers, has been demonstrated by multiple laboratories [135–137].

The injection of both cultured and freshly isolated ASCs into the peri-infarct area of the heart of mice has been identified to potentially improve cardiac function in experimentally induced myocardial injury. Two functional studies using in vivo infarction models were recently presented at the 2005 meeting of the International Fat Applied Technology Society (IFATS). In addition, cellular cardiomyoplasty appears hopeful in light of recent reports that mesenchymal stem cells produce cardioprotective factors in sufficient amounts to improve myocardial function within 72 h after cell transplantation [138]. This effect is confirmed by animal studies and clinical studies that treatment group has increased capillary densities [139, 140]. However, as smooth muscle differentiation, the in vivo differentiation of ASCs into cardiomyocytes is still controversial. Strem et al. reported that ASCs express cardiac markers in vivo 2 weeks after injection, but Cai and his co-workers [141] found that intramyocardially injected ASCs differentiated into smooth muscle cells but not into cardiomyocytes in rats.

The therapeutic potential of ASCs may be further enhanced through their effects on neovascularization. ASCs in culture are known to secrete proangiogenic factors, including vascular endothelial growth factor (VEGF), hepatocyte growth factor, and transforming growth factor-β [142]. Under hypoxic conditions, they have shown enhanced secretion of VEGF and of an unidentified antiapoptotic factor [142]. In vitro, endothelial differentiation of ASCs has likewise been described in Matrigel cultures. In addition to the characteristic tubelike structures, differentiated ASCs also expressed endothelial markers, such as CD31, Flk-1+, and von Willebrand factor [143, 144]. Earlier studies have demonstrated that the beneficial effects are mediated by angiogenic and antiapoptotic cytokines produced by mesenchymal stem cells [138]. In vivo results from a hind limb ischemia model demonstrated increased capillary density and perfusion in limbs treated with ASCs, further supporting the cells' angiogenic potential [142, 145]. These promising results may enhance the future application of ASCs in myocardial infarction, other cardiovascular diseases, and neovascular diseases that are one of the leading causes for morbidity and mortality in Western countries.

Muscular Disorders

Muscular dystrophies are a clinically and genetically heterogenous group of disorders characterized by progressive degeneration and loss of skeletal muscles [146]. Adult skeletal muscle has the potential to regenerate new muscle fibers by activating a population of mononucleated precursors, which otherwise remain in a quiescent and nonproliferative state [147]. However, the continuous and gradual muscle degeneration in progressive muscular dystrophies leads to depletion of satellite cells, and consequently, the capacity to restore the skeletal muscle is lost [148, 149]. Duchenne muscular dystrophy, an X-linked lethal disorder that affects 1 in 3–4,000 male births, is the most prevalent form of muscular dystrophy [150]. In case of muscle injury, muscle satellite cells are activated to become myogenic precursor cells. These cells divide and fuse to repair the damaged muscle. However, the mature muscle satellite cells represent 1–5 % of the total muscle cells and their potential for self-renewal decreases with age [151]. In DMD, the intense degeneration that occurs in muscle fiber exhausts the ability of satellite cells to proliferate and replace damaged fibers [149].

Initial efforts using myoblast transfer demonstrated short-term benefits, but were ultimately limited by poor cell survival, immune rejection, and poor migration of transplanted cells. There evolved the strategy to treat muscular diseases by restoring dystrophin levels in patients with DMD using cellular therapies. Those cell-based therapies that may replenish the exhausted supply of satellite cells may be particularly suited to prevent this decline. Several groups demonstrated that ASC is capable of differentiating along multiple lineages, including myocytes, in presence of lineage-specific induction medium [152]. This was demonstrated by the presence of certain characteristic markers and the formation of multinucleated myotubules [152–154]. Two hypotheses exist on the contribution of ASC to muscle regeneration: de novo generation of muscle-specific cells from ASC and modification in gene expression after direct fusion of ASC with host cells.

In the first in vivo study reported by F Bacou [155], ASCs were injected into the anterior tibialis muscle of rabbits following cardiotoxin-induced injury. Although this study was statistically significant, clinically noticeable improvements were uncertain. Other in vivo studies showed that implantation of ASC in mdx mice restored dystrophin expression in the dystrophic mouse cells [48]. Allogenic ASCs injected intravenously or directly into the affected muscle could restore muscle function in a murine muscular dystrophy model without any signs of immune rejection [156]. Another study, conducted by Kim and his co-workers [100], used PLGA spheres attached to myogenically induced ASCs to inject subcutaneously into athymic nude mice. Injected ASCs differentiated into muscle cells and regenerated new muscular tissue. Although the demonstration that ASCs have myogenic potential both in vitro and in vivo is promising, it is still unclear whether ASCs directly differentiate into myogenic lineage cells or they become incorporated into muscle fibers via cell fusion [203]. However, further work is needed to establish the methods necessary to treat progressive diseases.

Ectodermal Applications

Neurodegeneration

Neural tissue has long been regarded as incapable of regeneration, and the identification of stem cell population capable of neuronal differentiation has generated intense interest [157, 158] amidst the scientific community. The ability to differentiate into neuronal lineage is yet another property of ASC that provides evidence for its range of plasticity, much broader than originally thought. The ASC can achieve neurosphere formation when cultured at high density. Subsequent culture of the spheroid bodies on the laminin leads to final neural differentiation. Differentiated cells can be identified with the protrusion of extensive cell processes with a neuronal morphology consistent with a neural phenotype and expression of early neural precursors. Further trials have successfully induced the expression of an even broader range of mature neuronal and glial markers [159–162]. It remained as a controversy as to whether such neurons are electrically active and functional with all essential characteristics of mature CNS neurons. However, several in vivo studies carried out in animal model and humans, so far, had given maximum promising results for the treatment of cerebral ischemia, spinal cord injury, as well as neurological diseases [163]. ASC has also been identified to migrate into the injured cerebral lesions after ischemic stroke and has improved functional deficits and motor recovery. Despite major improvements, it is uncertain whether transplanted cells contribute to the direct replacement of lost neurons or provide a supportive role for existing in situ stem cells and injured neurons. In a coculture model, Kang and his coworkers [164] studied the interactions between neural stem cells and adipose-derived stem cells. In comparison to laminin-coated dishes, ASC feeder layers showed an ability to support the differentiation and survival of neural stem cells over 14 days in culture. The functional improvements seen after stem cell infusion in vivo may be a result of this supportive ability. Engraftment studies have demonstrated that ASCs cross the blood–brain barrier [155], but their role after they arrive is unclear. Although certain aspects of neural differentiation are unclear, it is important to note that evidence exists for functional improvements in ischemia, traumatic brain injury, as well as spinal cord injury using ASCs. However, further demonstrations on ASCs are required to reasonably prove the fact beyond doubt and to advance its applicability.

Endodermal Application

Liver Cirrhosis and Hepatic Failure

Most liver diseases lead to hepatocyte dysfunction with the possibility of eventual organ failure. Liver transplant may be the only option to treat patients with heavily damaged livers in case of both acute and chronic failures. The major barrier for this transplantation is the shortage of donors and immune rejection, which is still unsolved. Cell-based therapies have been under investigation for the past decade to fill this void of failure of treatment of hepatic dysfunction. The replacement of diseased hepatocytes by stem cells and the stimulation of endogenous or exogenous regeneration by stem cells are the main aims of liver-directed cell therapy. There is growing evidence of reports describing the hepatocytic differentiation potential of ASC [165–168] and the effective functions of these differentiated hepatocyte-like cells not only in vitro but also in vivo. In 2005, Seo and his co-workers reported that hASCs cultured in media supplemented with growth factors and cytokines yielded a cell population that expressed a number of hepatocyte-specific functions, such as albumin production and urea synthesis. A subsequent in vivo model tracked human ASCs injected into mice 2 days following exposure to a known hepatotoxin. Donor cells engrafted in the liver assumed a hepatocyte-like morphology and began expressing albumin [167]. Similarly, transplantation of hASCs into SCID mice with acute liver failure caused by CCl4 injection revealed that undifferentiated hASCs were able to engraft into the liver and improve its function [168]. Although these data suggest that transplanted cell may differentiate in vivo into mature hepatocytes, they did not address the rate of recovery of liver function, and the approach of using hASC is at its infancy. Plenty of issues still remain to be investigated before application is justified in clinical setting for enhanced tissue repair and recovery of organ failure.

Diabetes Mellitus

Diabetes is a major malady that causes a large portion of epidemic deaths worldwide. The epidemic proliferation of diabetes is at such a high rate that new drugs and other therapeutic approaches are required to curb it. Over the last few decades, the main therapeutic approach to insulin-dependent diabetes has confined to the use of insulin injections [169], but was proven ineffective in replacing normoglycemic level. As an alternative strategy, insulin gene therapy focuses on converting non-β-cells into insulin-producing cells by introducing insulin synthetic genes with secretary techniques [170, 171]. Transplantation of islets of Langerhans has been shown to be successful in experienced centers, but this therapy can be offered only to a very limited number of patients due to shortage of donors and immune rejection [172–176]. Regeneration of pancreatic β-cell has emerged as a recent advancement in stem cell technology. Insulin-secreting cells generated from stem cells could represent an attractive alternative.

Transplantation of pancreatic beta islet cells had showed that approximately 70 % of the patients treated for type I

diabetes achieved insulin independence [177]. Autologous islet transplantation (AIT) has proved to be an efficacious treatment strategy to prevent surgical diabetes. AIT has also proven efficacious in normalizing glycemic levels in patients with benign pancreatic tumors and pancreatic trauma [178]. AIT has been shown to be beneficial for diabetes treatment; the transplanted islets do not suffer allogenic rejection and diabetogenic antirejection drugs are not required. However, the limited availability of autologous pancreatic cells limits the applicability of this technique. Considering these prevailing conditions, autologous stem cells as well as autologous stem-/progenitor-derived insulin-secreting islet-like clusters are gaining importance. Transdifferentiation of subcutaneous adipose tissue-derived stem cells into beta cells has been carried out in vitro [81] using multistep differentiation procedure, providing direct evidence that human ASCs could be programmed to become functional insulin-producing cells. These generated islet-like clusters were confirmed to secrete insulin in response to glucose stimulation and express various molecules that resembled those expressed by pancreatic beta cells, such as Isl-1, Pax4, Pax6, pancreatic/duodenal homeobox 1 (Pdx1), prohormone convertase (PC) 1/3, PC2, Kir6.2, glucose transporter (Glut) 2, glucokinase (GK), as well as the islet gene insulin. As the numbers of diabetic patients are increasing in recent years, it is of utmost importance to focus more on stem cell-based therapy for diabetes treatment. In lieu of this, a few but exciting reports from clinical trials conducted at different parts of the world are highlighted. Adipose-derived stem cells have been used as a novel therapy for patients suffering type II diabetes, where autologous activated adipose-derived stem cells were given as intravenous or direct catheter injection into the pancreatic artery of the patients proving its efficacy and usefulness in diabetes treatment (NCT01453751). There have also been studies confirming the efficacy of intravenous administration of stromal vascular fraction as a treatment of type II diabetes (NCT00703612). These data have proven adipose tissue as a safe, efficient enormous source for stem cells for its use in diabetes treatment. However, further research into the factors and in vivo mechanisms of pancreatic development will augment ASC application in curative diabetes and diabetes-related disease treatment.

Other Applications

Crohn's Disease

Crohn's disease, a chronic bowel disease characterized by bloody stools, diarrhea, weight loss, and autoimmune-related symptoms, usually affects young people between the ages of 18 and 40 years. Conventional treatment using corticosteroids, immunosuppressant, and biological drugs for inflammatory complications with surgery or stenosis (narrowing of the intestinal lumen) or fistulas (openings from the intestinal lumen to other organs, such as the intestine, bladder, vagina, or skin) led to deteriorated quality of life. Therefore, treatment of this disease using innovative cellular therapy was a need and is promising as shown by a number of successful clinical trials [179, 200]. In a phase I trial with patients with fistulas unresponsive to standard treatment, cultured ASCs were directly injected into rectal mucosa, and 75 % of cases healed completely. In a phase IIb trial, the proportion of patients who achieved fistula healing was significantly higher with ASCs than with fibrin glue (Table 10.3).

Wound Healing

Several studies have evaluated the potential therapeutic effects of ASCs on wound healing. Local implantation of ASCs has been found to be effective in supporting epidermal healing in full-thickness skin wounds of pigs, as well as in rats, in which the survival area of ischemic skin flaps was significantly increased by local injection of autologous ASCs. Rigotti and his co-workers [180] reported successful results after injection of lipoaspirates containing ASCs to wounds caused by postmastectomy irradiation. According to ultrastructural analysis, the early stages of tissue mesenchymalization were observed after application of lipoaspirates, and a tissue resembling normal mature adipose tissue was formed at the site of application. The authors commented that the effect of lipoaspirate on wound healing is largely due to the angiogenic growth factors secreted by ASCs [181]. These results were valuable in terms of showing the safety and feasibility of ASCs for clinical wound management.

GVHD

Graft-versus-host disease (GVHD) constitutes the most frequent complication associated with the transplantation of allogenic cells. SVF and ASC both serve as an effective treatment for steroid-refractory acute graft-versus-host disease after allogenic transplantation owing to immunosuppressive property of MSC. It is being revealed from the immunosuppressive effect of MSCs in vitro and in preclinical animal models that MSCs may be used for the prevention and treatment of graft-versus-host disease (GVHD), in organ transplantation to prevent rejection, and in autoimmune disorders. Yanez and co-workers reported that adipose-derived stem cells do not generate in vitro alloreactivity of incompatible lymphocytes and suppressed the lymphocyte proliferative response to mitogens and alloantigens [182]. The study also demonstrates that MSCs obtained from third-party donors were well tolerated and exerted in vitro and in vivo

Table 10.3 An account on current clinical trials using SVF/ASCs

Conditions	Status	Phase	Treatment/mode of infusion	NCT number
Graft-versus-host disease; chronic and expanded Graft-versus-host disease; immune system diseases	Recruiting	Phase 1 Phase 2	Conventional treatment and intravenous infusion of allogenic MSC	NCT01222039
Autoimmune diseases; immune system diseases; demyelinating diseases; nervous system diseases; demyelinating autoimmune diseases, CNS	Recruiting	Phase 1 Phase 2	Intravenous infusion of autologous MSCs	NCT01300598
Degenerative arthritis	Recruiting	Phase 1 Phase 2	Intra-articular infusion of autologous MSCs	NCT01300598
Buerger's disease	Recruiting	Phase 1 Phase 2	Intramuscular infusion of autologous MSCs	NCT01302015
Articular cartilage lesion of the femoral condyle	Not yet recruiting	Phase 1 Phase 2	Implantation of autologous ASC or chondrocytes	NCT01399749
Osteoporotic fractures	Not yet recruiting	Phase 2	Cellularized and acellular composite graft augmentation	NCT01532076
Soft tissue mass removal	Completed	–	Liposuction	NCT01399307
Progressive hemifacial atrophy; Romberg's disease	Completed	Phase 2	Autologous MSC transplantation	NCT01309061
Leukemia; Hodgkin's lymphoma; non-Hodgkin's lymphoma; myelodysplastic syndrome	Active, not recruiting	–	Questionnaires, laboratory tests, abdominal MRI	NCT00510315
Rectovaginal fistula	Recruiting	Phase 1 Phase 2	Intralesional injection of cell suspension	NCT01548092
Anal fistula	Unknown	Phase 2	Nonsurgical autologous implant of ASCs	NCT00115466
	Completed	Phase 3	Intralesional injection of ASCs with fibrin glue	NCT00475410
Complex perianal fistula	Recruiting	Phase 2	Autologous cultured adipose-derived stem cells	NCT01314092
	Completed	–	Intralesional injection of ASCs with fibrin glue	NCT01020825
Crohn's fistula	Recruiting	Phase 1	Infusion of allogenic ASCs	NCT01440699
Crohn's disease	Recruiting	Phase 1 Phase 2	Autologous MSCs	NCT01300598
Frailty syndrome	Recruiting	Phase 1 Phase 2	Intravenous injection of MSCs	NCT01501461
Diabetes mellitus type II	Recruiting	Phase 1 Phase 2	Intrapancreatic injection; intravenous injection of the ASCs	NCT01453751
	Unknown	Phase 1 Phase 2	Intravenous injection of autologous activated SVF	NCT00703612
Diabetes mellitus type I	Unknown	Phase 1 Phase 2	Intravenous injection of autologous activated SVF	NCT00703599
Diabetes; limb ischemia	Recruiting	Phase 1 Phase 2	Intra-arterial infusion of autologous MSCs	NCT01079403
Lower limb ischemia	Recruiting	Phase 1 Phase 2	ASC-coated ePTFE vascular graft	NCT01305863
Parkinson's disease	Recruiting	Phase 1 Phase 2	Harvesting and implantation of ASCs (catheter injection)	NCT01453803
Brain lesion (general)	Recruiting	Phase 1 Phase 2	ASCs delivered via catheter into the internal carotid artery and intravenously	NCT01453777

(continued)

Table 10.3 (continued)

Conditions	Status	Phase	Treatment/mode of infusion	NCT number
Multiple sclerosis	Recruiting	Phase 1 Phase 2	ASCs delivered via intravenous injection and intrathecally	NCT01453764
Non-ischemic congestive heart failure	Recruiting	Phase 1 Phase 2	ASCs delivered intramyocardially and intravenously	NCT01502501
Stroke	Recruiting	Phase 1 Phase 2	ASCs delivered into the internal carotid artery and intravenously	NCT01453829
Renal failure	Recruiting	Phase 1 Phase 2	ASCs delivered via catheter into the renal artery and intravenously	NCT01453816
Depressed scar	Completed	Phase 2 Phase 3	Autologous cultured adipocytes via subcutaneous injection	NCT00992147
Lipodystrophy	Unknown	Phase 1	Lipoinjection enriched with ASCs	NCT00715546

immunoregulatory properties similar to those of autologous or allogenic MSCs [183, 184]. This opens new perspectives to the use of adipose MSCs for treating GVHD.

Autoimmune Diseases

Immunomodulatory properties appear to be an intrinsic property of ASC and thus present an attractive basis for the therapy of autoimmune and inflammatory diseases by systemic infusion. Treatments for autoimmune diseases were initiated in patients after other treatment options were exhausted. Recently, ASCs have been explored for its potential immunoregulation in autoimmune disease systemic lupus erythematosus (SLE) [185]. It is suggested that immunomodulation of ASCs was achieved by partially suppressing the number and capability of Th17 lymphocytes, indicating that ASCs could be employed as therapeutic tools for the autoimmune diseases. There is still a limited understanding of the modes of action of stem cells during the treatment of autoimmune diseases. Thus, systemic infusion of autologous stem cells might offer promise for better management of a wide spectrum of autoimmune diseases, independent on patient's age if further research on modes of action is explored.

Subcutaneous Adipose Stem Cell in Clinical Trial

Human subcutaneous adipose stem cells are abundant, accessible, and reliable source of therapeutic applicability in preclinical/clinical studies in diverse fields. It is rapidly increasing and gaining importance in recent years due to their ability to readily be expanded and their capacity to undergo multilineage differentiation and positive experimental data accumulated from the studies outlined above. The safety and efficacy of ASCs for tissue regeneration or reconstruction are currently under assessment in clinical trials. Although the clinical trials of BMSC crossed 500 in numbers completed or underway, clinical trials using ASC are less in number. However, the number of trials has risen rapidly from a total of 9 in December 2009, 18 by May 2010, to 32 in April 2012, investigating the efficacy in treating conditions such as type I and II diabetes, fistulas, cardiovascular disease, limb ischemia, depressed scar, lipodystrophy, and so on (http://clinicaltrials.gov) (Table 10.3). Furthermore, there are a limited number of currently ongoing phase III clinical trial investigating autologous ASCs in repairing perianal fistulas and depressed scar. Although the full publication of data from many of these trials is pending, early information from selected trials has been presented recently.

Conclusion

Regenerative medicine involves a multidisciplinary effort to replace or repair diseased tissue. Stem cell therapy and tissue engineering are the key components of regenerative medicine. However, both are linked by one common aim to deliver safe, effective, and consistent therapy to patients. Subcutaneous adipose depots are accessible, abundant, and replenishable and had accomplished these objectives, thereby providing a potential source as adult stem cell for treating a wide range of therapeutics. Several groups have demonstrated that mesenchymal stem cells within the human subcutaneous adipose tissue possess self-renewing ability and display multilineage developmental plasticity in vitro and in vivo. It is now becoming increasingly accepted that the likely predominant mechanism of action of ASC is survival, proliferation, and differentiation into tissue-specific stem cells, which then get incorporated into diseased tissues and repopulate the area with a healthy cell population thereby exerting an appreciable effect and promoting endogenous repair process.

Although our understanding of ASC biology is gradually increasing in recent years, many challenges lie ahead in aspects of the wealth of knowledge of all normal process and steps involving cell differentiation and tissue formation, before we can realize the full potential of these cells for clinical and therapeutic applications.

This chapter has highlighted some of the aspects of current important research investigations and key areas for future investigation which include the catalogue of cell surface marker characterization that enables the isolation of pure population of ASCs, prerequisites for ex vivo expansion studies, and optimization of culture condition, multilineage differentiation potential, and current registered clinical trials using ASC. The advances that have been observed with ASC have provided evidence of their great potential and applicability in cell therapy, as well as in the enhancement of healing process. Moreover, their reproducibility, easily handled characteristics, accessibility, and facility of being obtained in a relatively large quantity make them an ideal source of transplantation and a boost for regenerative medicine.

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