Chapter 14
Adipose-Derived Stem/Stromal Cells

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Abstract The adipose tissue is considered as a multifunctional organ which plays an important role in energy storage and endocrine and immune responses. In addition, it serves as a reservoir for a population identified as adipose-derived stem/stromal cells (ASCs). ASCs have been documented to possess the potential to differentiate toward multiple cell lineages both *in vitro* and *in vivo*. At present, 168 national and international clinical trials involving ASC have been registered according to the U.S. National Institutes of Health of which 38 have been completed. Both pre-clinical and clinical studies have shown the effectiveness of ASCs to treat various diseases. The mechanisms by which ASCs may provide regenerative function include their ability to differentiate into target tissue specific cells, the secretion of factors to recruit and direct host-derived reparative cells, and/or immunomodulatory effects. Thus, due to its abundance, easy availability, and low morbidity during harvest, adipose tissue provides a feasible tissue source of adult stromal/stem cells for regenerative medicine.

Keywords Adipose-derived stem cell • White adipose tissue • Brown adipose tissue adipocyte • Chondrocyte • Osteoblast • Secretome • Preconditioning

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14.1 Introduction

Historically, adipose tissue was considered exclusively as an energy reservoir. More recently, other adipose physiological functions such as metabolic regulation, inflammatory processes and endocrine function have been characterized. Additionally, human adipose tissue has been identified as an abundant and accessible source of multipotent stromal/stem cells. These cells have the potential to differentiate into cells of mesodermal origin including adipocytes, chondrocytes, osteoblasts, and myoblasts, as well as cells of non-mesodermal lineages, such as hepatocytes, pancreatic endocrine cells, neurons, cardiomyocytes, and vascular endothelial cells. The International Fat Applied Technology Society (IFATS) and the International Society for Cellular Therapies (ISCT) reached a consensus to adopt the term "adipose-derived stromal/stem cells" (ASCs) to identify the isolated, plastic-adherent, multipotent cell population obtained from adipose tissue (Bourin et al. 2013). Over the past decade and a half, ASCs have been widely studied in tissue engineering and regenerative medicine as well as cell therapy.

14.2 Classification and Derivation of Adipose

Adipose tissue is comprised of a heterogeneous cell population which includes adipocytes, endothelial cells, pericytes, and pre-adipocytes as well as various immune cells (Lee et al. 2013). Cells resembling mesenchymal stem cells (MSCs; also known as multipotent stromal cells) have been found within the adipose tissue which are now identified as ASCs. The ASCs are isolated by collagenase digestion, mechanical disruption and/or explant culture techniques. Anatomically, there are at least five different categories of adipose tissue: bone marrow, brown, mammary, mechanical, and white (Gimble et al. 2007). The ASCs isolated from different depot can display similar but not necessarily identical biological properties (Baglioni et al. 2012; Shah et al. 2015). Additionally, the biological characteristic of ASCs has been reported to differ between lean and obese as well as between young and old donors (Schipper et al. 2008). In the future, it may be necessary to determine whether a particular kind or depot source of ASCs is more suitable for therapy of a specific disease.

14.2.1 White Adipose Tissue

White adipose tissue (WAT) is recognized as a multifunctional organ displaying, for example, endocrine, energy storage, immunomodulatory, and secretory roles. Two of the most prominent WAT depots are the subcutaneous adipose tissue and the visceral adipose tissue. The WAT serves as an energy reservoir, efficiently storing excess calories in the form of triglyceride. Both hypertrophy and hyperplasia of adipocytes occurs when caloric input exceeds expenditure; in contrast, the stored

triglycerides are metabolized into free fatty acids and glycerol when energy is required (Otto and Lane 2005). The ability of WAT to expand extensively suggests the existence of stem cells and labeling studies indicate that adipose tissue contains long term label retaining cell *in vivo*, consistent with the existence of a stem-like cell (Gawronska-Kozak et al. 2014).

In recent years, liposuction has been employed for body sculpting. According to the American Society for Aesthetic Plastic Surgery Reports, 342,494 liposuction procedures were performed in the United States in 2014, making it among the most commonly performed cosmetic procedures. Up to 3 kg of fat can be acquired from this procedure, however the fat is routinely discarded as medical waste. Alternatively, using collagenase enzyme digestion or mechanical disruption, a stromal vascular fraction (SVF) can be isolated from the fat. After expansion *in vitro*, these cells become more homogeneous for a subset of surface antigen biomarkers and are identified as ASCs. In many respects, the ASCs meet the criteria defining human mesenchymal stem cells (MSCs). The ASC are able to differentiate along multiple lineage, including adipocyte, chondrocytes and osteoblasts. These cells express surface markers associated with the MSC immunophenotype. In comparison to bone marrow stem cells (BMSC), ASCs have a higher colony forming unit (CFU) frequency (Kern et al. 2006) and a higher cell yield per gram tissue (Strem et al. 2005). Therefore, the easy accessibility, abundant source, and limited harvest morbidity make subcutaneous WAT an exciting alternative to bone marrow as a stromal/stem cell source.

14.2.2 Brown Adipose Tissue

In humans, brown adipose tissue (BAT) is mainly located on the back and around the major organs in infants and in the cervical, supraclavicular, and superior mediastinal depots in adults (Cypess et al. 2009; Virtanen et al. 2009). BAT is characterized by an abundance of mitochondria expressing uncoupling protein-1 (UCP1) (Cannon and Nedergaard 2004). UCP1 is found in the inner membrane of brown adipocytes mitochondria where it is responsible for the non-shivering thermogenesis of BAT (Cannon and Nedergaard 2004). It has been well recognized that BAT shares a Myf5+ precursor with skeletal muscle, making BAT cells more closely related to skeletal muscle rather than WAT adipocytes. Recently, a population of Myf5+ precursor were found in interscapular WAT (iWAT) and retroperitoneal WAT (rWAT) (Sanchez-Gurmaches et al. 2012). The ASCs isolated from BAT have been found to express a similar cell surface marker profile and a multilineage potential resembling that of ASCs from WAT (Silva et al. 2014). This may reflect an ability of adipose tissue to transdifferentiate between WAT and BAT. Conversion of WAT to BAT is observed during shivering response/cold exposure, or through the enhanced expression of peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) or forkhead box protein C2 (FOXC2), agonists to peroxisome proliferator-activated receptor γ (PPARγ), retinoic acid or nicotine (Stephens et al. 2011). However, the BAT-like cells derived from WAT origin are described

currently as a unique sub-type known as beige/brite cells, which will be discussed in greater detail below. BAT exhibits a lower stromal/stem cell yield per weight of tissue and weaker differentiation ability than WAT-derived ASC (Prunet-Marcassus et al. 2006). By comparing Sca-1$^+$/CD45$^-$/Mac1$^-$ ASCs isolated from murine BAT, WAT, and skeletal muscle, investigators have found that ASCs isolated from BAT serve as constitutively committed brown fat precursors (Schulz et al. 2011). Additionally, CD29$^+$ ASCs isolated from BAT could differentiate into cardiomyocytes with a high efficiency relative to those from WAT (Yamada et al. 2006). When these CD29+ BAT-derived ASCs were transplanted *in vivo* along with chitosan hydrogel, they are able to preserve and repair myocardial tissue following myocardial infarction (Wang et al. 2014a). By injection of BAT-derived ASCs in high fat fed NOD-SCID mice, no change was found in body weight compared to the saline control animals (Silva et al. 2014). These results suggest that the BAT derived ASC hold potential for stem cell therapy and regenerative medicine.

Clinically, BAT tissue has been associated with disease types such as obesity, type 2 diabetes and insulin resistance, and atherosclerosis (Harms and Seale 2013). In mice which have been genetically modified to express less BAT there is an observed increase in weight gain, suggesting that there may be an inverse correlation between BAT depot volumes and obesity. Furthermore enhancing the activity or availability of BAT tissue in mice has correlated with resistance to weight gain and suppression of metabolic diseases such as type 2 diabetes (Harms and Seale 2013). Mice with enhanced BAT activity and presence have an increased sensitivity to insulin. BAT is known to be induced thermogenically as well as to be regulated and enhanced through a subset of genes, FOXC2, phosphatase and tensin homolog (PTEN), CCAAT-enhancer-binding protein β (C/EBPβ), and PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16). Likewise, there are many known inhibitors of BAT including activin receptor type IIB (ActRIIB), activating transcription factor 4 (ATF4), pre-adipocyte factor-1 (Pref-1), eukaryotic initiation factor 4E-binding protein-2 (4E-BP2), forkhead box-containing protein O subfamily 1 (FOXO1), transcriptional intermediary factor-2 (TIF2), and protein kinase Cβ (PKCβ) (Seale 2010).

14.2.3 Additional Adipose Depots

Adipose tissues are present throughout the body. In addition to BAT and WAT, adipose tissue has been found in the appendicular skeleton. The bone marrow fat is involved in thermogenesis, hematopoiesis, energy metabolism, and secretion of adipokines (Gimble et al. 1996a; Hardouin et al. 2014). Mammary adipose tissue acts as a local site for hormone action, the storage of lipids, and growth factor production (Hovey et al. 1999). The adipose depots located in the retro-orbital, buccal, palmar, and plantar regions provide mechanical support (Gimble et al. 2007). Further research will be needed to understand the specific biological properties of ASCs from these depots (Fig. 14.1).

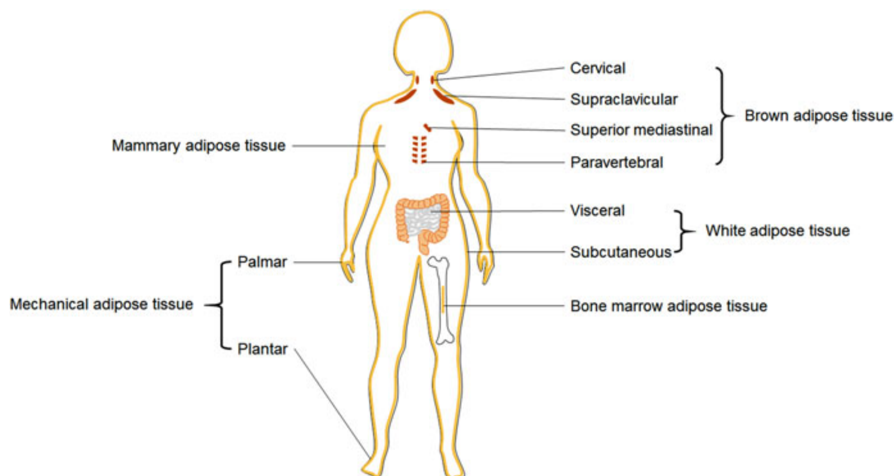

Fig. 14.1 Representative adipose depots

14.2.4 Beige Adipocytes

As stated above, there are instances of brown adipose like cells in WAT and these cells are designated as "beige" or "brite" (brown-in-white) adipocytes (Petrovic et al. 2010). While UCP1 has been used as a definitive biomarker for brown adipocytes, studies using northern analysis and PCR analysis detected UCP1 unexpectedly in WAT (Cousin et al. 1992). After cold exposure or β3-adrenoceptor agonist treatment, the expression of UCP1 increased in WAT in rodents (Cousin et al. 1992). Beige adipocytes were enriched within the inguinal WAT of mice. Beige adipocytes markers including CD137, transmembrane protein 26 (TMEM26), transcription factor T-box 1 (Tbx1) and Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1 (Cited1) have been reported (Wu et al. 2012; Sharp et al. 2012). Early B-cell factor 2 (Ebf2) is selectively expressed in brown and beige adipocytes (Wang et al. 2014b). The Asc-type amino acid transporter 1 (Asc-1), proton assistant amino acid transporter-2 (Pat2), and purinergic receptor P2X, ligand-gated ion channel 5 (P2rx5) have been proposed to serve as distinguishing cell surface markers for white, beige, and brown adipocytes, respectively (Ussar et al. 2014). Studies showed that the beige adipocytes in mice are originally derived from Myf5^{-}PDGFRα^{+} precursor cells (Sanchez-Gurmaches et al. 2012). Meanwhile, white adipocytes can transdifferentiate into beige adipocytes on a second cold stimulation (Rosenwald et al. 2013). In light of these recent studies, the cellular origin of beige, brown and white adipocytes is still under investigation (Bartelt and Heeren 2014; Harms and Seale 2013; Wu et al. 2015).

14.3 Immunophenotype of Adipose-Derived Stem/ Stromal Cells

The Mesenchymal and Tissue Stem Cell committee of the ISCT has outlined the minimal criteria for defining the human mesenchymal stem/stromal cells: (1) Plastic-adherent when cultured under standard conditions; (2) The expression of CD105, CD73 and CD90 must ≥ 95 %, while, on the other hand, the expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and human leucocyte antigen (HLA) class II must ≤ 2 %. (3) The cells must be capable to differentiate into adipocytes, chondroblasts and osteoblasts (Dominici et al. 2006). More recently, the same committee extended its original guidelines by incorporating an evaluation of MSC immunomodulatory function based on mixed lymphocyte reactions and related assays (Krampera et al. 2013).

Similar recommendations from IFATS and ISCT have been proposed for ASCs (Bourin et al. 2013). The ASCs share a great majority of cell surface markers expression with MSCs. The freshly isolated SVF are heterogeneous with expression of low levels of stromal-associated markers such as CD13, CD29, CD44, CD73, CD90, CD105, and CD166. After *in vitro* expansion, high levels of stromal marker expression have been observed in ASCs (Mitchell et al. 2006). The human ASCs are consistently positive for these surface proteins: CD9, CD10, CD13, CD29, CD34, CD44, CD49e, CD54, CD55, CD59, CD90, CD105, CD166 and leucocyte-associated antigens-ABC (HLA-ABC), but negative for CD11a, CD11b, CD11c, CD14, CD18, CD31, CD45, CD49d, CD50, CD56, CD62e, and HLA-DR (Gronthos et al. 2001; Aust et al. 2004). In addition to those markers, studies have reported that ASCs are also positive for CD51, CD71, STRO-1 but negative for CD4, CD8a, CD16, CD41a, CD49f, CD62L, CD62P, CD104, CD106, CD117, CD133, CD243 (Katz et al. 2005; Zuk et al. 2002). However, the expression of CD49b, CD49d, CD61, CD138, and CD140a showed a variable positivity among different donors (Katz et al. 2005). The surface markers expression suggests that ASCs display a similar profile as MSCs.

Studies have documented the immunophenotype change after differentiation. After adipogenic differentiation, the ASCs expression of CD36, CD40, CD146, CD164, and CD271 has been reported to increase while CD49b, CD49c, CD49d, CD71, CD105, and CD166 were decreased. Following osteogenic differentiation, CD164 expression has been found to be up-regulated but CD49a, CD49b, CD49c, CD49d, CD55, CD58, CD105, and CD166 expression was reported as down-regulated (Walmsley et al. 2015).

Efforts have been made to purify the ASCs. Using fluorescence-activated cell sorting (FACS) technique, a subpopulation of Lin − CD29 + CD34 + Sca-1 + CD24+ cells has been sorted out from the mice SVF. These cells are thought to be undifferentiated adipocyte precursor (Rodeheffer et al. 2008). In human, both CD31+ and CD31− SVF are capable of adipogenic differentiation; of these, the CD31− cell population are more robust (Wosnitza et al. 2007). In one study, human CD34− ASCs have been reported to display a stronger adipogenic differentiation capacity

than CD34+ ASCs (Suga et al. 2009). In contrast, CD45−CD31−CD34+ cells sorted from human SVF were reported to represent a subpopulation with an inherent propensity for adipogenesis exceeding that of other subpopulations (Li et al. 2011). Consistent with this observation, the CD34 biomarker has been associated with volume retention in human adipose tissue fat grafts, suggesting that CD34 positivity is a potential biomarker for adipogenic progenitors or stem cells (Philips et al. 2013). Additionally, the CD10 and CD200 expression have been demonstrated to be depot-dependent and are correlated with adipogenic differentiation potential (Ong et al. 2014). Likewise, CD105− ASCs have been found to show stronger adipogenic differentiation ability relative to their CD105+ ASCs counterparts; however the osteogenic and chondrogenic differentiation potential is more robust in CD105+ ASCs than in CD105− ASCs (Jiang et al. 2010). In summary, cells with distinctive lineage differentiation capacity can be sorted by using different combination of surface markers. It remains to be determined if the ASCs contain, in addition, a true stem cell.

14.4 Differentiation of Adipose-Derived Stem/Stromal Cells

As highlighted above, there is evidence from multiple independent laboratories demonstrating that ASCs are multipotent, with adipogenic, osteogenic, and chondrogenic differentiation capability *in vitro* and *in vivo*.

14.4.1 Adipogenic Differentiation

Adipogenic differentiation is one of the hallmark characteristics identifying ASCs. A complex cascade of transcriptional and extracellular signals have been determined to regulate the adipogenic differentiation process (Rosen and MacDougald 2006). The master transcription factors of adipogenesis are PPARγ and the C/EBPs. To study the adipogenic process, ASCs are exposed to a defined adipogenic cocktail which routinely includes dexamethasone (DEX), insulin, and methylisobutylxanthine. Dexamethasone is a synthetic glucocorticoid agonist, which is used to stimulate the glucocorticoid receptor pathway. Insulin acts at the level of the extracellular insulin receptor, initiating a complex cytoplasmic and nuclear response. Methylisobutylxanthine is a cAMP-phosphodiesterase inhibitor which elevates cytoplasmic cAMP levels, thereby activating the cAMP-dependent protein kinase pathway (Ntambi and Young-Cheul 2000). Other adipogenic reagents have been utilized in the adipogenic cocktail such as indomethacin or thiazolidinedione (a ligand for the PPARγ) and triiodothyronine, a ligand for the thyroid receptor, but an adipogenic response can occur in their absence (Gimble et al. 1996b; Hauner et al. 1989). To confirm adipogenic differentiation histochemically, Oil Red O or Nile Red staining is routinely used to detect intracellular lipid accumulation (Yu et al.

2011; Williams et al. 2011). Adipogenic markers such as PPARγ, C/EBPα, lipoprotein lipase, leptin and adipocyte protein 2 can be determined at the mRNA and protein level. The robust adipogenic capacity of ASCs makes it a valuable cell model for human metabolism and obesity studies as well as a resource for regenerative medicine.

14.4.2 Osteogenic Differentiation

To induce osteogenesis in stromal/stem cells, an osteogenic cocktail is used. In general the composition of this cocktail contains β-glycerophosphate (β-GP), DEX, and ascorbate 2-phosphate. The β-GP is hydrolyzed by alkaline phosphatase (ALP) to produce high levels of phosphate which promotes mineral deposition. DEX acts as a ligand for the glucocorticoid receptor and activates *Runt-related transcription factor (Runx2)*/core-binding factor subunit alpha-1 *(CBF-alpha-1)* expression, a transcription factor integral to osteogenesis. In addition DEX also acts to induce activation of the WNT/β-catenin and mitogen-activated protein kinase (MAPK) phosphatase (MKP-1) pathways. Ascorbic acid enhances the formation of the collagenous bone extracellular matrix (Langenbach and Handschel 2013). In addition to extracellular calcium deposition, differentiated osteoblasts have increased ALP activity which can be measured using commercially available kits. Alizarin Red or Von Kossa staining is frequently used to determine the formation of calcium deposits (Bourin et al. 2013). The molecular osteogenic markers such as Runx2, ALP, osteocalcin (OCN), osteopontin (OPN), and bone sialoprotein (BSP) can be detected via PCR and western blot after osteogenic differentiation. Expression of these genes is temporal where early induced osteogenic genes include Runx2 followed by genes associated with matrix mineralization such as OCN. Due to the abundance and osteogenic capacity of ASCs, the cells has been used for bone regeneration (Lendeckel et al. 2004; Sandor et al. 2014).

14.4.3 Chondrogenic Differentiation

To induce chondrogenesis in ASCs, a chondrogenic cocktail containing ascorbate 2-phosphate, dexamethasone, ITS (insulin, transferrin, and selenium), transforming growth factor beta 3 (TGF-β3) and bone morphogenetic protein 6 (BMP6) is often used (Erickson et al. 2002; Estes et al. 2010). Ascorbic acid serves to promote increased protein and proteoglycan synthesis (Awad et al. 2003). The DEX, acting as a glucocorticoid ligand, is variably used in chondrogenic differentiation, but its effect may be context dependent (Shintani and Hunziker 2011). ITS is suitable as a partial or full substitute for serum in chondrogenic culture (Kisiday et al. 2005). Although TGF-β1 has been used in chondrogenic differentiation, other TGF-β superfamily member such as TGF-β2, TGF-β3, BMP2 and BMP-6 have also been

shown to modulate chondrogenesis (Kramer et al. 2000; Luo et al. 2015; van Osch et al. 1998). For chondrogenesis. ASCs can be cultured as either high-density pellet cultures or in three-dimensional calcium alginate compositions (Izadpanah et al. 2006; Erickson et al. 2002). Histochemically, Alcian Blue, Safranin O and Toluidine Blue staining are used to monitor the accumulation of sulfated proteoglycans. Likewise, immunohistochemistry staining of collagen type I and II has been used to identify active chondrogenesis. Expression of chondrogenic differentiation markers including aggrecan, collagen type I and II, cartilage oligomeric matrix protein/thrombospondin-5 (COMP/TSP5) and Sox9 can be evaluated via PCR and western blot analysis. The chondrogenic potential suggest ASCs is a feasible cell source for cartilage regeneration (Erickson et al. 2002).

14.4.4 Other Lineages Differentiation

Myogenic Differentiation Exposure of ASCs to myogenic medium can induce their differentiation to myocyte lineage pathways (Mizuno et al. 2002). This has suggested the possibility that ASCs can be used in skeletal muscle regeneration in combination with appropriate biomaterials (Kim et al. 2006).

Neurogenic Differentiation ASCs are able to undergo neural differentiation using different combinations of growth factors such as basic fibroblast growth factor (bFGF), glial growth factor-2 (GGF-2) and platelet-derived growth factor (PDGF) and regent like forskolin or retinoic acid (Kingham et al. 2007; Lopatina et al. 2011). The potential usage of ASCs in neural regeneration has also been reported (Lopatina et al. 2011).

Endothelial Differentiation The culture of ASCs using an endothelial differentiation medium which contains vascular endothelial growth factor (VEGF) and bFGF in combination with a Matrigel coating has led to the formation of an *in vitro* vascular-like network. Under these conditions, the ASC were positive for endothelial cell markers including CD31 (PECAM), CD34, and CD144 (VE-cadherin). Based on these findings, ASCs hold the potential therapeutic use in the treatment of ischemic diseases (Cao et al. 2005) and phase I clinical studies to treat ischemia of the lower extremities are now underway (Bura et al. 2014).

Hepatogenic Differentiation Using a two-step differentiation protocol, both BMSCs and ASCs can be differentiated toward hepatocytes. These results suggests that ASCs may serve as an alternative for hepatocyte regeneration (Talens-Visconti et al. 2006).

Tendonogenic Differentiation Growth differentiation factor-5 (GDF-5) treatment has been reported to induce tendonogenic gene expression in rat ASCs (Park et al. 2010a). Furthermore, the use of tendon-derived extracellular matrix can enhance the tenogenic differentiation and may facilitate tendon healing and regeneration (Yang

et al. 2013). Thus, there is the potential to apply ASCs in the treatment of ligamentous defects.

Keratinocyte Like Cells Either by co-culture with human keratinocytes or by exposure to the keratinocyte-derived conditioned media from, ASCs can transdifferentiate into keratinocyte-like cells *in vitro* (Chavez-Munoz et al. 2013). The development of large numbers of keratinocytes would have potential benefit in the treatment of patients with third degree burns covering a large percentage of their body surface area.

14.5 Secretome

The secretome is comprised of the secreted proteins and microRNAs from a cell, tissue, or organ at any given time and under particular growth conditions. The role of the secretome has been noted in light of the reparative effects of transplanted mesenchymal stem cells despite their failure to display long term differentiation and/or engraftment within a damaged tissue. Further mechanistic studies have determined that paracrine factors may be responsible for the enhanced regeneration within the pathologic tissues and organs. Since stromal/stem cells communicate with each other via autocrine and paracrine pathways, it has been hypothesized that such secreted factors play an important role in mediating the multiple biological functions of stromal/stem cells during regeneration, such as cell proliferation, differentiation, apoptosis and signaling.

Numerous studies in the literature have documented that the ASC secretome can exert benefits on angiogenesis, tissue regeneration, wound healing and immunomodulation. The pro-angiogenic effects of the ASC secretome has been correlated with the secretion of angiogenic factors such as VEGF, bFGF, hepatocyte growth factor (HGF), granulocyte colony stimulating factor (G-CSF) and TGF-β (Rehman et al. 2004). The secretome from ASC has been reported to promote endothelial cell survival, proliferation, migration, and vasculogenesis (Merfeld-Clauss et al. 2015). The ASC secretome may promote wound healing by inducing the endothelial cell migration since it exerts this effect on human umbilical vein endothelial cells *in vitro* (Hu et al. 2013). The ASC conditioned media has also been found to increase skin allograft survival (Lee et al. 2014). Likewise, the soluble factors from ASC have shown promise for the treatment of photo-aging (Kim et al. 2009). Additionally, treatment of alopecia patients with adipose-derived stem cell-conditioned medium led to effective hair regeneration (Fukuoka and Suga 2015). The ASC secretome can also alleviate liver damage (Lee et al. 2015b) and enhance the liver regeneration in mice (Lee et al. 2015c). The ASC secretome has been demonstrated to induce bone regeneration in surgically created lesions in a lupine mandibular defect (Linero and Chaparro 2014). The ASC secretome exhibited neuroprotective effects against a pre-clinical stroke mice model (Egashira et al. 2012). Recent studies suggest that the ASC secretome acts mechanistically by inhibiting neuronal cell damage/apop-

tosis, thereby promoting nerve regeneration and repair (Hao et al. 2014). In related studies, ASC have been found to promote peripheral nerve regeneration partly through paracrine secretome effects (Sowa et al. 2012). The ASC conditioned media is able to induce chondrogenesis at a high concentration, due, in part, through TGF-β related signaling pathways (Kim et al. 2010). The ASC conditioned media has been found to promote keratinocyte differentiation via the up-regulation of miR-24 (Seo et al. 2015). While the secretome from ASCs has shown immunomodulatory effects, the cytokines, nitric oxide (NO), and indoleamine 2,3-dioxygenase (IDO) production have been found to vary between different mouse strains (Hashemi et al. 2013). Thus, the ASC secretome has a promising and wide-ranging potential for clinic application and translation.

Nevertheless, while the secretome of ASC has potential benefits to regenerative medicine, the secreted factors themselves are present in relatively low amounts (picogram scale) (Rehman et al. 2004). Efforts have been made to isolate and characterize the components of the secretome by proteomics technologies. Using two-dimensional gel electrophoresis and tandem mass spectrometry, over 80 individual protein have been identified after adipogenic differentiation (Zvonic et al. 2007). Cytokine arrays and liquid chromatography coupled with tandem mass spectrometry were also utilized to analyze the secretome (Kapur and Katz 2013). Future studies will need to develop methods to enrich the relevant proteins or factors in the ASC secretome to insure its utility in regenerative medicine.

14.6 Preconditioning of the Adipose-Derived Stem/Stromal Cells

ASCs therapy has shown promising results in regenerative medicine. However, limited survival has been noted after cell transplantation (Fan et al. 2013; Suga et al. 2014). Cell death is caused by their exposure to a severe microenvironment within the pathologic or reconstructed tissues (Robey et al. 2008). Thus, a variety of preconditioning strategies have reported to enhance the therapeutic effect of MSCs including biological, chemical and physical manipulation (Haider and Ashraf 2010; Sart et al. 2014). These strategies are primarily designed to enhance the pro-angiogenic effects, improve the survival of the ASC and direct cells along a specific lineage pathway.

Biological Preconditioning In this respect, biological macromolecule including growth factors, cytokines, and polysaccharide have been used for preconditioning of ASCs. Erythropoietin preconditioning significantly suppressed ASCs apoptosis (Ercan et al. 2014). Preconditioning with tumor necrosis factor-alpha (TNF-a) increased proliferation, mobilization, and osteogenic differentiation of ASCs (Lu et al. 2013). PDGF-D preconditioned ASCs showed an increase in their proliferation and migration, which further enhanced hair regenerative ability (Hye Kim et al. 2015). Lipopolysaccharide (LPS) pre-conditioning, acting via the toll-like receptor

4 (TLR4), stimulated ASCs to produce a secretome beneficial to hepatic regeneration (Lee et al. 2015a). Betancourt and her colleagues have demonstrated that brief exposure to TLR3 (poly di/DC) or TLR4 (LPS) ligands can promote an anti- or pro-inflammatory phenotype in ASC as well as MSC (Bunnell et al. 2010). Exendin-4 is an antidiabetic polypeptide hormone and its preconditioning is able to prevent ASCs apoptosis (Zhou et al. 2014). Platelet-rich plasma (PRP) contains several growth factors and treating ASCs with PRP is able to promote proliferation and chondrogenic differentiation but inhibit angiogenic factor secretion (Van Pham et al. 2013). These methods may prove of value in future regenerative medical approaches.

Chemical Preconditioning Chemical conditioning of ASCs has included both chemical reagents and drugs. Stromal/stem cells are typically cultured under atmospheric air oxygen tensions of 21 %. However, mesenchymal stem cells *in vivo* reside at lower oxygen tensions of 2 %–8 % (Mohyeldin et al. 2010). Oxygen tension have been found to regulate the differentiation, proliferation and metabolism of ASCs (Wang et al. 2005; Malladi et al. 2006). Extracellular matrix (ECM) protein production by ASCs was decreased by 5 % oxygen tension exposure (Frazier et al. 2013). Insulin-like growth factor binding protein (IGFBP)-1, IGFBP-2, macrophage colony-stimulating factor (M-CSF), M-CSF receptor, platelet-derived growth factor receptor-beta (PDGFR-β), and VEGF secretion in ASC was significantly increased by low oxygen tension exposure (2 %) (Park et al. 2010b). Furthermore, 2 % oxygen tension pre-conditioned ASCs promoted hair growth in mice (Park et al. 2010b) and exhibited increased proliferation and enhanced their wound-healing function (Lee et al. 2009). In addition to oxygen tension manipulation, there are alternative pre-conditioning regimens to consider. Vitamin C preconditioning enhanced the hair growth promoting effect of ASCs (Kim et al. 2014). Studies have found that ascorbate induced expression of HGF in ASCs (Kilroy et al. 2007). Phosphodiesterase-5 (PDE-5) inhibitor sildenafil preconditioning enhanced the therapeutic effect of ASCs for myocardial infarction (Hoke et al. 2012). VEGF secretion was increased by deferoxamine preconditioning, which may enhance their therapeutic efficacy (Liu et al. 2013). Low-dose rapamycin treatment promoted ASCs viability (Fan et al. 2013). Preconditioning ASCs with a mixture of hyaluronic, butyric, and retinoic acids were able to increase the secretion of angiogenic factors (Cavallari et al. 2012). VEGF and HGF secretion was improved and apoptosis was prevented by rotenone and antimycin pretreatment (Carriere et al. 2009). In general, chemical preconditioning primarily improved the pro-angiogenic effects of ASCs.

Physical Preconditioning Physical and mechanical preconditioning of ASCs has also been performed. These approaches including mechanical, sound, electrical, magnetic, light and heat stimulation. Mechanical force has been documented to direct stem cell fate (Estes et al. 2004). Studies found that mechanical properties of three-dimensional biomaterials played an important role for cartilage regeneration (Awad et al. 2004). Research showed that shear stress generated by medium flow

enhanced osteogenic differentiation of ASCs (Frohlich et al. 2010). In addition to mechanical preconditioning, low-intensity ultrasound treatment enhanced cartilage regeneration when BMSCs were seeded into polyglycolic acid following *in vivo* transplantation (Cui et al. 2006). Meanwhile, low-intensity pulsed ultrasound enhanced the osteogenic differentiation of ASCs *in vitro* (Yue et al. 2013). Direct current electrical stimulation of ASCs enhanced the expression of connexin-43 (Cx-43), VEGF, FGF, and thrombomodulin (ThB) (Tandon et al. 2009). In addition to this, electric stimulation enhanced the osteogenic differentiation of ASCs *in vitro* (McCullen et al. 2010). Electromagnetic field preconditioning of ASCs resulted in a significantly better bone regeneration in mouse model (Kang et al. 2012). Low dose of Ultraviolet (UV) B radiation was found to increase the survival, the migration, and secretion of angiogenic factors in ASCs, which further promoted enhanced hair regeneration (Jeong et al. 2013). It has been documented that heat shock preconditioning was able to improve Sca-1+ BMSCs survival (Feng et al. 2014) and enhanced the osteogenic differentiation of BMSC-based cell lines (Norgaard et al. 2006). It will be worthwhile to pursue the effects of physical preconditioning in future *in vitro* and *in vivo* experiments.

14.7 Clinical Applications of Adipose-Derived Stromal/Stem Cells

A number of clinical trials using freshly isolated SVF cells and ASCs are ongoing. Commercial ASCs separation systems are available for clinical usages (Aronowitz and Ellenhorn 2013). By searching on the U.S. National Institutes of Health's website (https://clinicaltrials.gov/), 168 studies were found under the search term of "adipose stem cell" (as of July 1st, 2015). The majority of studies are registered in East Asia (41), Europe (46), and North America (51) (Fig. 14.2).

Soft Tissue The adipose tissue origin of ASC allows its use for adipose tissue reconstruction to be defined unequivocally as "homologous use". Cell assisted lipotransfer (CAL) involves the combination of freshly isolated autologous SVF with the injection of intact lipoaspirate tissue. In early reports on the procedure, the vast majority of the 40 patients receiving CAL for cosmetic purposes were satisfied with the appearance and texture of their augmentation (Yoshimura et al. 2008). At postoperative 12 weeks, patient and doctor surveys confirmed that the results of CAL treatment were significantly better than those grafted with adipose tissue without SVF cells (Lee et al. 2012). It has been reported that CAL techniques have the clinical advantage in reducing the graft resorption at one year (Domenis et al. 2015). Similarly, fat grafts enriched with high dose *ex-vivo* expanded ASCs (20×10^6 cells per mL fat) retained more than 80 % of the initial bolus volume 4 months after the grafting procedure (Kolle et al. 2013). Thus, both SVF and ASCs display potential utility for soft tissue regeneration.

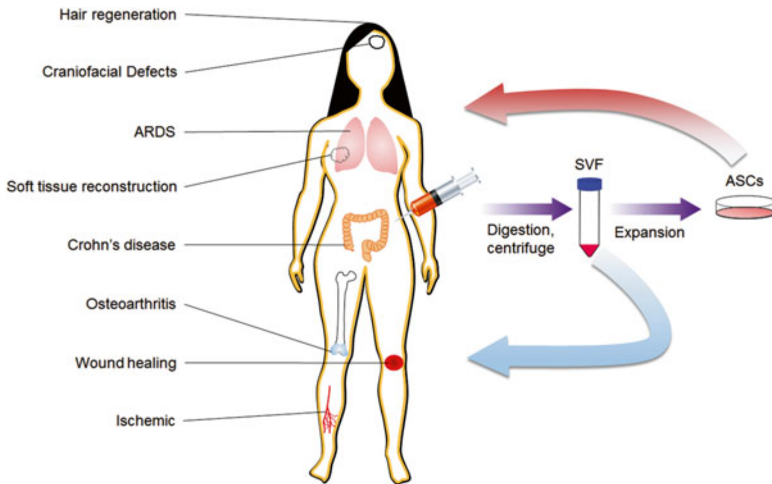

Fig. 14.2 Representative clinical applications of SVF/ASCs

Wound Healing Injection of autologous SVF treated the acute complications of skin necrosis, resulting in much less scarring (Sung et al. 2012). The ASC-based therapy to modulate scar formation has begun to receive particular attention in Korea.

Bone and Cartilage Regeneration Studies suggest that ASCs are an alternative cell source for bone and cartilage regeneration. Scaffolds seeded with autologous ASCs have been used to successfully reconstruct cranio-maxillofacial hard-tissue defects in a series of 13 patients (Sandor et al. 2014). Injection of autologous SVF intra-articularly or peri-articularly was found to improve osteoarthritis symptoms in a total of 1,128 patients after 3–12 months (Michalek et al. 2015). Likewise, injection of ASC into the osteoarthritic knee joint improved function and pain of the knee joint without causing adverse events, and reduced cartilage defects by regeneration of hyaline-like articular cartilage in 18 patients (Jo et al. 2014).

Ischemic As discuss above, ASC secretes a large variety of angiogenic factors which suggested a potential application for ischemic diseases. Intramuscular injection of ASCs was found to improve revascularization and tissue perfusion in ischemic limbs of seven patients (Bura et al. 2014). In a similar study, 10 patients showed variable degrees of recovery by injection of SVF into the edges of ischemic ulcers (Marino et al. 2013).

Crohn's Disease Phase I, II and III clinical trial have documented that both autologous and allogeneic ASCs combined with fibrin glue are an effective and safe treatment for complex perianal fistula (Garcia-Olmo et al. 2005, 2009; de la Portilla et al. 2013; Herreros et al. 2012). ASCs injection combined with thrombin and fibrinogen also showed a complete healing in Crohn's patients (Cho et al. 2013) and

complete closure was well-sustained after 2 years (Cho et al. 2015). Furthermore, such cell therapy did not compromise patient fertility or pregnancy outcomes (Sanz-Baro et al. 2015).

Hair Regeneration Injection of conditioned medium collected from ASCs significantly increased the hair numbers in patients (Fukuoka and Suga 2015; Shin et al. 2015). The secretion of HGF, IGFBPs, M-CSF, PDGF-β, and VEGF might account for the hair follicle promoting effects (Park et al. 2010b; Won et al. 2010).

Others ASCs continue to receive considerable attention from investigators pursuing adult stem cells clinical applications in a variety of disorders. For example, ASCs have been used for the treatment of acute respiratory distress syndrome (Zheng et al. 2014) and a phase I clinical trial used ASCs to serve as carriers for an oncolytic measles virus therapy (Mader et al. 2013). It is likely that many investigators will examine the utility of ASC in multiple disease states; however, it is imperative that a strong mechanistic rationale be presented to regulatory authorities for the use of ASC before initiating any such studies.

14.8 Future Perspectives

Due to the abundance and great potential in stem cell area, ASCs have been received attention from multiple basic science and clinical disciplines. In addition to the direct usage of SVF/ASC/ ASC secretome in multiple disorders, studies have found that ASCs may be an ideal autologous cell source for reprogramming induced pluripotent stem cells (iPS) cells (Sun et al. 2009). ASCs can also serve as an effective therapeutic gene delivery vehicle (Kucerova et al. 2007). There is a growing body of evidence from independent, international laboratories documenting the pre-clinical and clinical safety and efficacy of ASC therapies. To advance the future clinical translation of ASC, it will be necessary to: (1) establish a Good Manufacturing Practice (GMP) standard for clinical cell isolation; (2) characterize the functional difference of ASC from different depots; (3) evaluate the safety and effectiveness of allogeneic ASC; (4) monitor the behavior of ASCs when they are transplanted *in vivo*, and, last but not least; (5) perform long term follow up on the intended and un-intended consequences in patients receiving ASC and SVF cell therapies.

Disclosure J.M. Gimble is the co-owner, co-founder and Chief Scientific Officer of LaCell LLC, a for-profit biotechnology company focusing on the development of stromal/stem cell research tools and their use for clinical translation.

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