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REVIEW

Adipose-derived stem cells: Implications in tissue regeneration

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Abstract

Adipose-derived stem cells (ASCs) are mesenchymal stem cells (MSCs) that are obtained from abundant adipose tissue, adherent on plastic culture flasks, can be expanded *in vitro*, and have the capacity to differentiate into multiple cell lineages. Unlike bone marrow-derived MSCs, ASCs can be obtained from abundant adipose tissue by a minimally invasive procedure, which results in a high number of cells. Therefore, ASCs are promising for regenerating tissues and organs damaged by injury and diseases. This article reviews the implications of ASCs in tissue regeneration.

Key words: Mesenchymal stem cells; Adipose-derived stem cells; Differentiation; Growth factors; Tissue engineering; Clinical trials

Core tip: This review article provides an overview on adipose-derived stem cells (ASCs) for implications in tissue regeneration. ASCs are obtained in high yields from abundant adipose tissue in the body and have multi-lineage differentiation ability. This article focuses on ASC characterization, growth factor secretion from ASCs, differentiation ability *in vitro* and *in vivo*, and the potential clinical applications.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are adult stem cells that were originally identified in bone marrow as multi-potent cells[1,2]. Stem cells are characterized by their self-renewal ability and multi-potency. Bone marrow-derived stem cells are most broadly studied for therapeutic potentials since their discovery in the 1960s[1]. After the discovery of bone marrow-derived MSCs, MSCs have been isolated from nearly every tissue in the body[3], for example, adipose tissue[4], umbilical cord blood[5], peripheral blood[6], dental pulp[7], dermis[8], and amniotic fluid[9], and even in tumors[10]. Adipose-derived stem cells (ASCs) were first identified as MSCs in adipose tissue in 2001[11], and since then adipose tissue has been studied as a cell source for tissue engineering and regenerative medicine. There are multiple terms for stem cells derived from adipose tissue, for example, preadipocytes, adipose-derived stromal cells, processed lipoaspirated cells, adipose-derived mesenchymal stem cells, adipose-derived adult stem cells. In 2004, the consensus was reached the term as ASCs.

There are several types of adipose tissue, with subcutaneous as the most clinically relevant source. ASCs can be isolated from subcutaneous adipose tissue of the abdomen, thigh, and arm. Because adipose tissue is typi-

cally abundant in the human body, ASCs can potentially be isolated in high numbers. The multi-lineage capacity of ASCs offers the potential to repair, maintain or enhance various tissues. This review article will focus on source, isolation, and characterization of ASCs, secretion of growth factors from ASCs, *in vitro* and *in vivo* differentiation ability of ASCs, and the potential clinical application.

SOURCE, ISOLATION, AND CHARACTERIZATION OF ASCS

There are mainly two types of adipose tissue: white adipose tissue and brown adipose tissue. They are morphologically and functionally different. Brown adipose tissue much less abundant than white adipose tissue, but can be found in the neck, mediastinum, and interscapular areas in neonates. However, brown adipose tissue undergoes a morphologic transformation with aging. The appearance of brown adipose tissue is literally brown. Brown adipocytes are multilocular and retain small lipid vacuoles compared to white adipocytes. Vascularization is obvious because brown adipose tissue requires much more oxygen consumption compared to other tissues. Brown adipocytes have no known correlation with insulin resistance. The main function of brown adipose tissue is thermogenesis[12,13]. Brown adipose tissue contains a large number of mitochondria and expresses uncoupling protein 1 (UCP1). UCP1 is a brown adipose tissue-specific marker, not expressed within white adipose tissue. UCP1 is expressed in the inner membrane of mitochondria, mainly regulated by adrenergic signaling through sympathetic innervations, and this signaling is responsible for thermogenesis[12,13]. Brown adipose tissue is activated by thyroid hormone, cold temperatures, thiazolidinediones, and activated brown adipose tissue is inversely correlated with body mass index, adipose tissue mass and insulin resistance.

White adipose tissue is found throughout the body, representatively in subcutaneous and visceral adipose tissue. The appearance of white adipose tissue is yellow or ivory. White adipocytes are unilocular and contain large lipid vacuoles. White adipose tissue function is to store excess energy in the form of triglycerides, and its hyperplasia causes obesity and dysfunction of metabolic pathways as insulin resistance. UCP1 is not expressed in white adipocytes but the isoform UCP2 is expressed in parts of white adipocytes.

Recently, beige adipocytes have been discovered within white adipose tissue, especially inguinal white adipose tissue[14]. Beige adipocytes have the characteristics of both brown and white adipocytes. Beige adipocytes contain both unilocular large and multiple small lipid vacuoles. Its function is adaptive thermogenesis. In response to cold temperature exposure, beige cells transform into cells which have brown adipose tissue-like characteristics, such as UCP1 expression and small lipid vacuoles[15]. It is still controversial whether the beige adipocytes arise through the transdifferentiation of white adipocytes or by *de novo* adipogenesis from a subgroup of precursor cells[16,17].

ASCs isolated from white adipose tissue have different characteristics from those isolated from brown adipose tissue, just as ASCs from different anatomical areas have different characteristics. Subcutaneous tissues are easily obtained *via* lipoaspiration and usually discarded after the surgery. The lipoaspiration technique does not affect function of ASCs, but the vacuum process does damage mature adipocytes[18].

Zuk *et al*[4] developed a widely used method for isolating ASCs from white adipose tissue in 2001. Adipose tissues are minced and then undergo enzymatic digestion with collagenase type Ⅱ. After centrifugation, the resulting pellet is called the stroma vascular fraction (SVF). Approximately 2 to 6 million cells in SVF can be obtained from one milliliter of lipoaspirate[19]. SVF contains ASCs, endothelial cells, endothelial progenitor cells, pericytes, smooth muscle cells, leukocytes, and erythrocytes[20]. ASCs are obtained as the plastic-adherent population after overnight culturing.

Stem cell yield is higher from adipose tissue than bone marrow-both for aspirated and excised adipose tissues. One gram of aspirated adipose tissue yields approximately 3.5×10^5 to 1×10^6 ASCs. This is compared to 5 hundred to 5×10^4 of bone marrow-derived MSCs (BM-MSCs) isolated from one gram of bone marrow aspirate[21]. However, ASC yield from lipoaspirated adipose tissue has been reported to be approximately one half that isolated from whole, excised adipose tissue[22]. ASCs are isolated from the SVF after plating, as ASCs adhere fairly quickly to the surface of tissue culture-treated flasks. ASCs are easily cultured and expanded *in vitro*; average doubling time of cultured ASC varies between 2 to 5 d, depending on passage number and culture medium[23,24]. ASCs can be easily cryopreserved in a media containing serum and dimethylsulfoxide. Proliferation and differentiation of ASCs are not affected by cryopreservation[25]. The morphology of ASCs is spindle-shaped, very similar to BM-MSCs.

One notable characteristic of ASCs is that they are not homogenous population[11,26]. Many studies have attempted to characterize ASCs using cell surface markers *via* flow cytometry analysis, but a unique single marker has yet to be identified. ASCs have a positive expression of CD34 at the first passage of culture, but CD34 expression decreases after passaging[20,23]. ASCs express typical mesenchymal markers such as CD13, CD29, CD44, CD63, CD73, CD90, and CD105, and ASCs are negative for hematopoietic antigens such as CD14, CD31, CD45, and CD144[4,23,27]. After culturing and passaging, ASC's surface markers can change with passaging. The expression of hematopoietic markers such as CD11, CD14, CD34, and CD45 dissipates or are lost[28]. On the other hand, the expression level of CD29, CD73, CD90, and CD166 increase from the SVF to passage 2[23]. Passaging is considered to select cell population with more homogenous cell surface markers compared to SVF.

Further characterization of the heterogeneous ASC

population has been recently reported. Li *et al*[26] categorized four ASC subpopulations: pericytes as $CD146^+/CD31^-/CD34^-$, mature endothelial cells as $CD31^+/CD34^-$, premature endothelial cells as $CD31^+CD34^+$, and preadipocytes as $CD31^-/CD34^+$. The highest subpopulation was preadipocytes with 67.6%, premature endothelial cell was the second highest subpopulation with 5.2%, and the percentage of pericytes and mature endothelial cells were less than 1%. The cells with $CD31^-/CD34^+$ expression demonstrated the greatest proliferation and highest adipogenic differentiation. The localization of ASCs within adipose tissue is not totally clarified yet, but the niche of ASCs is suggested to be in the vasculature of adipose tissue[29]. Histological analysis also suggested that ASCs reside within adipose tissue in a perivascular location[30,31]. Traktuev *et al*[31] concluded that the location of the ASCs in the vessel is at interface between endothelium and adipocytes, and ASCs have the ability to both support vascular structure and generate adipocytes.

ASC GROWTH FACTOR SECRETION

ASCs are considered to be a mediator of tissue regeneration through the secretion of specific soluble factors. ASCs secrete multiple growth factors, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1, hepatocyte growth factors (HGF), and transforming growth factor (TGF)-β1[32]. The expression of cytokines renders ASCs promising therapies for transplantation and ischemia patients. Transplanted tissues and organs are exposed to hypoxia soon after transplantation due to a lack of initial vasculature and tend to undergo apoptosis[33]. The levels of VEGF, bFGF, and HGF secreted by ASCs are reported to be upregulated by hypoxia. VEGF was secreted at the concentrations of 70.17 and 200.17 pg/mL under normoxia and hypoxia, respectively. Basic FGF was secreted at the concentrations of 10.62 and 24.75 pg/mL under normoxia and hypoxia, respectively[34]. Similarly, Rehman *et al*[35] reported that ASCs secreted significant levels of VEGF and HGF under hypoxia, which induced the healing mice hindlimb ischemia. ASCs are considered to be a cell source that induces angiogenesis, which is actually used for human ischemia treatment[36].

In addition to growth factor secretion, ASCs are responsive to growth factors, including enhancing proliferation by bFGF, and platelet-derived growth factor (PDGF). Basic FGF is released from an injured extracellular matrix[37]. PDGF is released from activated platelets on bleeding[38]. When ASCs are exposed growth factors, tissues can be regenerated more effectively. Kaewsuwan *et al*[39] studied effect of six growth factors on the proliferation of ASCs, and found that PDGF-BB had the highest stimulatory effect at the concentration of 10 ng/mL. PDGF receptors α and β are expressed in ASCs, and PDGF-BB and PDGF receptor β signaling is involved in the stimulation of ASCs[39]. Besides PDGF receptors α and β, ASCs express VEGF, HGF, epidermal growth factor (EGF), and bFGF receptors[40,41]. VEGF increases migration and promotes chondrogenic differentiation[41], and HGF promotes hepatogenic differentiation of ASCs *in vitro*[42]. EGF inhibits ASC adipogenic differentiation, and bFGF increase ASC proliferation, promotes adipogenic and chondrogenic differentiation, and inhibit osteogenic differentiation *in vitro*[43-47].

ASCs possess unique paracrine characteristics. ASCs secrete growth factors that stimulate recovery of damaged tissue. Furthermore, ASCs express several kinds of growth factor receptors and are sensitive to growth factors. Therefore, ASCs mediate tissue regeneration.

IN VITRO DIFFERENTIATION ABILITY OF ASCS

ASCs can be differentiated into multiple lineages under culturing with specific conditions[11], which results in the potential of ASCs for multiple clinical applications. The induction of ASC differentiation *in vitro* is achieved by culture with media containing selective lineage-specific induction factors. ASCs have been shown to be differentiated into cells of ectodermal, endodermal and mesodermal origin[4,48,49]. Less controversial is the differentiation of ASCs into adipogenic, chondrogenic, and osteogenic cells, because ASCs are of mesodermal origin. With a combination of morphological observation, immunofluorescence, and polymerase chain reaction (PCR) analysis *in vitro*, adipogenic, osteogenic, and chondrogenic potentials of ASCs has been reported[4,11]. As mentioned above, MSCs from different anatomical sources demonstrate some differences. ASCs have prominent adipogenic differentiation ability compared to BM-MSCs *in vitro*[50,51]. BM-MSCs have been shown to have higher osteogeneic differentiation ability compared to ASCs[51-53].

ASCs can be differentiated into adipocytes when cultured in adipogenic differentiation media, which typically contains isobuthyl-methylxanthine, insulin, and indomethacin[54]. ASCs develop multiple lipid droplets about 7 d following exposure to the induction media, and the number of lipid droplets gradually increases. By 2 to 3 wk, the lipid droplets begin to form a unilocular lipid. During differentiation into mature adipocytes, ASCs express several types of extracellular matrix (ECM) proteins, including fibronectin, laminin, and various types of collagen. During adipogenesis, a fibronectin network develops first, and a type- I collagen network is formed last[55]. These ECMs allow ASCs to differentiate into mature adipocytes. ASCs show promise for soft-tissue applications. Lipid droplets contain triglycerides, and can be easily confirmed histologically using Oil red O and Sudan Ⅲ staining. Gene expression that is specific to mature adipocutes includes peroxisome proliferator activated receptor (PPAR)-γ2, leptin, aP2, and glucose transporter type 4[56]. The real-time PCR study showed that the expression levels of PPAR-γ2 in ASCs isolated from female mice were higher than in those from male mice, suggesting that adipogenic differentiation of ASCs is closely related to gender[57].

When ASCs are cultured in osteogenic differentiation media, which may contain 1,25-dihydroxyvitamin D3, ascorbate-2-phosphate, and bone morphogenetic protein-2 (BMP)-2, for 2 to 4 wk, the cells differentiate into osteoblast-like cells *in vitro*[58]. After differentiation, the osteoblast-like cells start to produce calcium phosphate within the ECM which can be assessed with Alizarin Red or von Kossa staining to reveal osteocytes. Alkaline phosphatase, type I collagen, osteoponin, osteocalcin, bone sialoprotein, Runx-1, BMP-2, BMP-4, parathyroid hormone receptor, BMP receptor 1 and 2 are common genes that are up-regulated during osteogenesis[56]. Furthermore, male ASCs differentiate into bone more rapidly and more effectively than female ASCs[59].

For chondrogenic differentiation, cells typically require a 3D environment, such as an "aggregate culture" or "micromass pellet culture". The micromass pellet culture model mimics precartilage condensation during embryonic development, which increases the cell-to-cell interaction and leads to the production of a cartilage-like matrix[60]. Chondrogenic differentiation requires the use of a defined media supplemented with TGF-β1, insulin, dexamethasone, ascorbate-2-phosphate, and BMP-6. Basic FGF can be used to expand ASCs, and at the same time, down-regulate chondrogenic markers during cell expansion[61]. Differentiated chondrocytes express type Ⅱ collagen, type Ⅳ collagen, aggrecan, prolyl endopeptidase-like, and sulfate-proteoglycan[62]. Alcian blue and collagen type Ⅱ staining indicate chondrocytes.

Although somewhat controversial, ASCs may possess ectodermal differentiation capacity, *e.g.*, neurogenesis. Many studies have been reported[48,63,64]. Under culture conditions with media containing butylated acid, valproic acid, and insulin, ASCs become morphologically similar to neurons, and express markers of both neuronal (neuron-specific enolase, nestin, and NeuN) and glial lineages [S100, p75, nerve growth factor (NGF), receptorm, and NG2][48]. The differentiation of ASCs into Schwann cells that are capable of myelinating peripheral neurons has been reported[48]. Human ASCs form nestin-positive neurospheres and express Schwann cell markers including S100, glial fibrillary acidic protein, and the p75 NGF receptor after dissociation.

In addition to mesodermal and ectodermal capacity, the endodermal differentiation of ASCs has been reported. Numerous studies reported differentiation of ASCs into hepatocytes and beta islet cells[42,65,66]. In an environment with the differentiation factors activin-A, exendin-4, HGF, and pentagastrin, ASCs were demonstrated to differentiate into insulin-producing cells *in vitro*[66]. Meanwhile, adding HGF, oncostatin M, and dimethyl sulfoxide in the culture media resulted in the ability of ASCs to gain hepatocytic functions *in vitro*, including albumin and alpha-fetoprotein expression and urea production[42].

IN VIVO DIFFERENTIATION ABILITY OF ASCS

While *in vitro* differentiation of ASCs into multiple phenotypes has been reported, the *in vivo* translation can be challenging. While ASCs have many advantages as a cell source, (*e.g.*, easily harvested, abundant, and easy to culture), there remains the challenge of cell survival *in vivo*. Poor cell survival after *in vivo* injection or implantation is common. This is in part due to the hypoxic environment, particularly if cells are transplanted into ischemic tissues. ASCs have been shown to survive in ischemic tissues, whereas mature adipocytes die easily under ischemic conditions[67], ASCs also secrete angiogenic factors under hypoxic conditions[34,35]. For certain clinical applications, ASC implantation may require suitable biomaterial scaffolds that support cell attachment, proliferation, and differentiation. The scaffold should be selected based numerous characteristics, such as porosity, bioactivity, mechanical integrity, biodegradability, and low immunogenicity. Ideal scaffolds can provide cells with an environment suitable for cell survival[68]. The environment immediately following implantation can be severe for the cells to survive because oxygen and nutrients are insufficient. Implanted cells need to survive until effective angiogenesis occurs. As described above, ASCs secrete significant levels of angiogenic factors under hypoxia. ASCs can survive in an ischemic environment, and provide a reservoir of growth factors that are necessary for angiogenesis.

ASCs have immense potential in wound healing applications. Altman *et al*[69] grafted an acellular dermal matrix construct seeded with human ASCs into a murine injury model, and found that ASCs enhanced the wound healing at day 7. Most of the ASCs were viable 2 wk after the engraftment. An appropriate scaffold contributed to ASC homing and surviving. ASCs grafted in the wound can result in the augmentation of the local blood supply and in an improvement of regeneration capacity.

As ASC differentiation into adipocytes is well established, adipose tissue regeneration using ASCs *in vivo* has been investigated. Clinical applications include soft tissue augmentation after injury, surgical resection, and congenital malformations. Among the strategies to generate adipose tissue are the combination of ASCs and scaffolds, the use of acellular scaffolds, and the addition of drugs or growth factors to the scaffolds that have been examined include type I collagen, fibrin, silk fibroin, alginate, hyaluronic acid, and matrigel[70-72]. Injectable scaffolds are an attractive option, as minimally invasive therapies would be widely adapted by surgeons. Methods of drug delivery include using polymeric microspheres to control the release of factors such as bFGF, insulin, and dexamethasone[73-75].

Regarding osteogenic potential, ASCs show promise for bone tissue regeneration after injection or congenital malformations. Since ASCs were discovered to have osteogenic potential, many *in vivo* studies have combined ASCs with biodegradable scaffold materials to promote bone growth. Immuno-deficient animal models for nonweight-bearing bone formation have become a common model to assess human ASC osteogenic potential *in vivo*. Because bone is composed of hydroxyapatite (HA)

crystals, bioceramics such as HA and beta-tricalcium phosphate are used for bone regeneration. The ceramic biomaterials used in these applications should mimic the natural bone architecture to ensure ASC attachment and migration within porous materials. In addition, ceramic biomaterials should be absorbed overtime or integrated with the surrounding tissue and eventually replaced by new or existing host tissue. As collagen is the other main component of bone tissue, it has been widely studied as a natural biomaterial scaffold for bone regeneration. In contrast, synthetic polymers such as poly (L-lactic acid-co-glycolic acid), and poly-e-caprolactone (PCL), can also be utilized for bone engineering. The advantage of these polymers is that they are readily reproducible, and have flexible mechanical, chemical, and biological properties that allow them to be tailored to suit specific functions[76].

There has been much interest in examining ASCs for the cartilage tissue engineering required to remedy osteoarthritis (OA), which affects millions of patients all over the world. A cure for OA remains elusive, because, in part, while the cartilage ECM is maintained by a sparse population of chondrocytes, it exhibits little capacity for self-repair owing to the lack of a tissue blood supply. Researchers have investigated a variety of scaffold materials including alginate, agarose, fibrin, gelatin, and chondroitin sulfate to evaluate their ability to support chondrogenic differentiation of ASCs *in vivo*[77-79]. Several studies have demonstrated that ASCs were able to differentiate into chondrocytes *in vivo* when seeded within any of these scaffolds, but different construct materials can significantly influence the differentiation of ASCs and functional properties of the tissue-engineered construct[77,78].

Finally, ASCs also have potential in neural applications. Peripheral nerves can be regenerated if injuries are small, and bioengineering strategies are focused on alternatives to the nerve autograft[80]. Properties of the ideal nerve conduit should include biodegradability, controlled release of growth factors, incorporation of support cells, electrical activity, intraluminal channels, and oriented nerve substratum. Santiago *et al*[81] was among the first to report that implanted ASCs into the lumen of PCL-based nerve conduits in a rat sciatic nerve defect model was shown to promote the formation of a more robust nerve. However, both the endodermal and ectodermal transdifferentiation of ASCs remains to be validated.

CLINICAL APPLICATIONS

A number of clinical applications using ASCs can be found through searches and on clinical trial websites. ASCs are mainly used for cell-based therapy, and the combination of ASCs with biomaterials or drugs is still to be studied. Most studies use adipose tissue as the scaffold. Garcia-Olmo *et al*[82-86] performed phase Ⅰ-Ⅲ clinical trials to investigate the efficacy and safety of expanded ASCs in the treatment of complex perianal fistulae including Crohn's disease. Autologous ASCs were mixed with fibrin glue then injected into the fistulous tract. As a result, patients who received ASCs demonstrated a better rate of healing compared to the patients who received fibrin glue without ASCs. ASCs with fibrin glue therapy were determined to be a safe and effective for treating complex perianal fistulae. Two mechanisms of ASCs to treat fistulae are speculated: one was that ASCs induced immunosuppressive activity, and the other was that ASCs might help healing through the expression of matrix proteins[83].

One of the first clinical reports using stem cells derived from adipose tissue in a patient was reported in 2004. Lendeckel *et al*[87] reported a case of a 7-year-old girl suffering from widespread calvarial defects after severe head injury with multifragment calvarial fractures. This is among the first reports of bone tissue engineering using autologous stromal vascular fraction and fibrin glue, although it was a case study. Fibrin glue was manufactured from the patient's plasma 2 d prior to the surgery. SVF was kept in place using autologous fibrin glue, and computed tomography scans showed new bone formation 3 mo after the reconstruction. It was noted that ASCs have a great advantage in the point of cell yield compared to BM-MSCs especially for pediatric patients. Indeed, 295×10^6 mononuclear cells were extracted from 42.3 g adipose tissue, and about 2%-3% of these cells are expected to be stem cells[87].

The disadvantages associated with the implantation of synthetic materials or autologous fat grafts could be overcome by engineered adipose tissue. Stillaert *et al*[88] attempted adipose tissue engineering in 12 volunteers. Hyaluronic acid-based scaffolds were implanted in the subumbilical area with and without ASCs. Unlike successful results with nude mice[89], the hyaluronic acid-based scaffolds didn't support ASC survival and were not inductive towards adipose tissue formation in humans. Meanwhile, ASC enriched lipotransfer has been studied for facial lipoatrophy and breast augmentation[90,91]. Yoshimura *et al*[92] enrolled 15 patients, transplanted SVF containing lipoaspirate after removing artificial breast implants, and followed for 12 mo. It was concluded that ASC-rich lipotransfer is effective to enhance the volume of injected adipose tissue[90-92]. The increased volume of adipose tissue may not be due to ASC differentiation but paracrine support of the tissue through the secretion of angiogenic and adipogenic factors. However, the interaction between ASCs and cancer cells are not fully elucidated. ASCs may promote cancer growth and metastasis through paracrine properties, epithelial-mesenchymal transition[93,94], and immunosuppressive mechanisms[95,96]. Higher risk of local recurrence was observed in early stage breast cancer patients following lipoinjection[97]. ASCs have not only bright side for regenerative medicine but dark side as cancer promotion.

In the field of wound healing, Rigotti *et al*[98] showed ASCs are effective on severe symptoms such as atrophy, retraction, fibrosis, or ulcers induced by radiation therapy. Twenty patients were recruited and received lipoaspirate containing ASCs repeatedly, and followed-up to 31 mo. Patients demonstrated an improvement of ultrastructual

tissue characteristics with neovessel formation as well as significant clinical improvements. The authors concluded that the treatment with ASC-containing lipoaspirates is potentially extended to other forms of microangiopathies.

Regarding the potential of ASCs to generate immune tolerance for transplant patients, ASCs have been reported to have an immunomodulatory effect[99]. It has been shown that ASCs don't possess human leucocyte antigen class Ⅱ antigens, and ASCs can suppress inflammatory cytokines, stimulate anti-inflammatory cytokine interleukin-10, and induce antigen-specific regulatory T cells[100]. In a case study, the intravenous infusion of allogenic ASCs in treating severe refractory acute graft-versus-host disease has proven to be effective[101]. Fang *et al*[102,103] treated patients with hematologic and immunologic disorders such as idiopathic thrombocytopenic purpura and refractory pure red cell aplasia, with allogenic ASC infusions, and reported significant improvements with these patients. From these results, ASCs are suggested to have immunomodulatory.

CONCLUSION

ASCs have prominent implications in tissue regeneration due to their high cell yield in adipose tissue, the ability to differentiate into multiple lineages and secrete various cytokines, and immunomodulatory effects. A large number of clinical trials using ASCs have already performed and many of them are ongoing. However, very few phase Ⅲ clinical studies have been published. ASCs are a promising cell source for regenerative medicine, and more research is needed to warrant the safety of ASCs and the efficacy of tissue engineering using ASCs.

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