

Review

STEM CELLS FROM ADIPOSE TISSUE

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Abstract: This is a review of the growing scientific interest in the developmental plasticity and therapeutic potential of stromal cells isolated from adipose tissue. Adipose-derived stem/stromal cells (ASCs) are multipotent somatic stem cells that are abundant in fat tissue. It has been shown that ASCs can differentiate into several lineages, including adipose cells, chondrocytes, osteoblasts, neuronal cells, endothelial cells, and cardiomyocytes. At the same time, adipose tissue can be harvested by a minimally invasive procedure, which makes it a promising source of adult stem cells. Therefore, it is believed that ASCs may become an alternative to the currently available adult stem cells (e.g. bone marrow stromal cells) for potential use in regenerative medicine. In this review, we present the basic information about the field of adipose-derived stem cells and their potential use in various applications.

Key words: Adult stem cells, Adipose-derived stem cells/stromal cells, Adipose tissue, Regenerative medicine

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Abbreviations used: ASCs – adipose-derived stem/stromal cells; BAT – brown adipose tissue, BM-MSCs – bone marrow mesenchymal stem cells; BMP – bone morphogenetic protein; ES – embryonic stem; HGF – hepatocyte growth factor; HSCs – hematopoietic stem cells; IGF – insulin growth factor; Il – interleukin; M-CSF – macrophage colony stimulating factor; MSCs – mesenchymal stem cells; Runx2 – runt-related transcription factor 2; SVF – stromal-vascular cell fraction; TGF- β 1 – transforming growth factor- β 1; TNF α – tumor necrosis factor α ; WAT – white adipose tissue; VEGF – vascular endothelial growth factor

INTRODUCTION

Regenerative medicine supports the natural healing processes in the reconstruction of tissues and organs by creating conditions in which the damaged or missing tissue can fully rebuild. The developed strategies include the transplantation of stem cells, the manipulation of the patient's own cells, and the use of scaffold materials that trigger biological signals in order to accelerate the regenerative processes. The successful application of innovative therapies has been confirmed in clinical trials and experiments assessing the healing of broken bones, severe burns, blindness, deafness, and heart, blood vessel, nerve and muscle damage, and in the treatment of many other diseases [1]. However, the availability of stem cells remains a challenge for scientists and clinicians. Ideally, stem cells for regenerative medical applications should be found in abundant quantities, harvestable in a minimally invasive procedure, then safely and effectively transplanted to either an autologous or allogenic host.

Stem cells are characterized by their ability to produce self-renewing progenitor cells that can generate one or more specialized cell types. Historically, stem cells are subdivided into two groups: embryonic stem cells (ES) and postnatal stem cells (i.e. hematopoietic stem cells as the prototypical example, umbilical cord blood cells, and adult somatic stem cells) [2]. Tumorigenicity and ethical considerations have impeded the widespread use of embryonic stem cells in clinical applications. Adult stem cells have aroused a much greater interest.

Every cell in the body ages and has a specific half-life. Changes in the body over time are due to the normal cell turnover. Such sequential replacement of cells in tissues and organs suggested the existence of progenitor cells that replace mature, older differentiated cells of complex tissues and organs. These progenitor cells are referred to as adult stem cells. It has long been believed that tissue-specific progenitor cells can only differentiate into native tissue cell types. Recent studies have challenged this view. Many experiments have revealed that adult stem cells may retain the potential to transdifferentiate from one phenotype to another, either *in vitro* or after transplantation *in vivo* [3, 4].

Until the year 2000, the focus of publications in the field of adult stem cells was limited to the hematopoietic stem cells (HSCs), bone marrow mesenchymal stem cells (BM-MSCs) and muscle satellite cells. Perivascular cells, principally pericytes, have been identified in multiple human organs, including the skeletal muscles, pancreas, adipose tissue and placenta. Perivascular cells from a variety of tissues exhibit a phenotype that is strikingly similar to that of MSCs derived from bone marrow, and they have multilineage mesodermal potential. Thus, blood vessel walls harbor a reserve of progenitor cells that may be integral to the origin of the elusive mesenchymal stem cells and other related adult stem cells [5]. The scope of current research includes the search for new sources of stem cells (dental pulp, hair follicles, amniotic fluid), and the investigation of their biology and potential therapeutic applications [6].

STEM CELLS FROM ADIPOSE TISSUE

The existence of stem cells within adipose tissue was reported for the first time in 2001 [7]. Adipose tissue is a type of connective tissue which is found under the skin (subcutaneous fat), around internal organs (visceral fat), in bone marrow (yellow bone marrow) and in breast tissue. In humans, it comprises one of the largest tissue types (at least 4% of adult human body mass), and it is central to the regulation of energy balance. Adipose tissue is found in two different forms: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the primary site of triglyceride storage, while BAT is specialized for energy expenditure and can counteract obesity [8]. In addition, adipose tissue acts as an endocrine organ that secretes numerous polypeptides, hormones such as leptin, resistin, and cytokines like Tumor Necrosis Factor α (TNF α) [9]. Furthermore, adipose tissue is probably one of the richest sources of adult stem cells in the human body, and thus it holds great promise for use in tissue repair and regeneration.

Adipose tissue is derived from the mesenchyme, and may be an alternative and minimally-invasive source of mesenchymal stem cells for regenerative medicine. These cells can be isolated from cosmetic liposuctions in large numbers, approximately $\sim 1 \times 10^6$ /200 ml fat [10]. Liposuction yields from 100 to 3000 ml of fat tissue [11]. This material is routinely discarded. The increasing number of obesity cases and plastic surgeries performed to remove fat tissue also results in a greater availability of the material for research into the use of isolated adipose stem cells for therapeutic applications in regenerative medicine. Thus, ASCs meet the important criteria for stem cells in regenerative medicinal applications: adipose-derived stem/stromal cells (ASCs) can be isolated (i) in significant numbers from fat tissue, (ii) without associated pain for the patient, and (iii) more easily than other types of multipotent mesenchymal stem cells (MSCs), such as bone marrow, placenta, amniotic fluid, and umbilical cord cells. Thus, adipose tissue could be an abundant, practical and appealing source of donor tissue for autologous cell replacement.

A variety of names have been used to describe the multipotent, plastic-adherent cell population isolated from adipose tissue. They are often described as processed lipoaspirate cells (PLA), preadipocytes, or adipose stem cells [12]. A fraction of stem cells, isolated by an enzymatic digestion of adipose tissue with collagenase [13] corresponds to the stromal-vascular cell fraction (SVF). The adipose stromal-vascular cell fraction is a heterogeneous mixture containing endothelial cells, preadipocytes, fibroblasts, vascular cells, macrophages, and numerous mesenchymal stem cells. These multipotent mesenchymal stem cells can give rise to several cell lineages. The International Fat Applied Technology Society recommend the term, "adipose-derived stem/stromal cells" (ASCs or ADSCs) [14] to refer to them.

Mesenchymal stromal/stem cell characterization is based on the expression of cell-specific proteins and CD markers. In 2006, the Mesenchymal and Tissue

Stem Cell Committee of the International Society for Cellular Therapy proposed a minimal set of four criteria for the identification of human mesenchymal stem cells [15], namely: (i) they have to be plastic-adherent when maintained under standard culture conditions; (ii) they must have the ability for osteogenic, adipogenic, and chondrogenic differentiation; (iii) they must express CD73, CD90, CD105; and (iv) they must lack the expression of hematopoietic lineage markers (CD14, CD11b, CD34, CD45, CD19, CD79).

The key characteristics of ASCs as stem/stromal cells include: the ability to adhere to plastic to form fibroblast-like colonies; an extensive proliferative capacity; and the ability to express several common cell-surface antigens [16]. They also possess the capacity to differentiate into several mesodermal lineages, including bone, muscle, cartilage and epithelium, as well as neural progenitors [17]. Many similarities between adipose-derived and other mesenchymal stem cells (i.e. bone marrow derived MSCs, umbilical cord blood cells) have been found. They concern both the morphology and the immunity phenotype, as well as the multipotency of the MSCs [18-21, 21, 22]. The initial adherent cells grow into spindle-shaped or stellate cells after the second passage, adopting a fibroblast-like shape. Intracellular lipid droplets have not been noted from the adipocytes. Such a morphology of ASCs was also observed in our laboratory (Fig. 1).

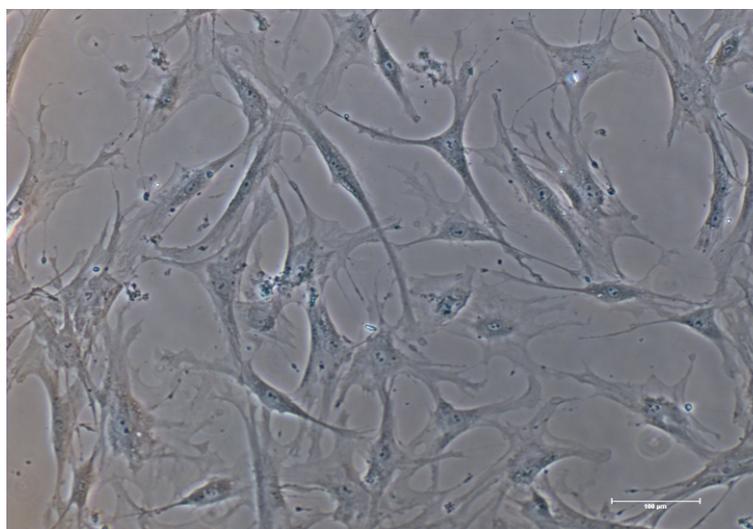


Fig. 1. Morphology of human ASCs cultured *in vitro*.

Microarray analysis and Real-Time PCR showed that BM-MSCs and ASCs exhibit a virtually identical transcriptional profile for stem-related genes [23, 24]. Therefore, it is believed that not only bone marrow, but also adipose tissue might be a suitable source of MSCs. Given that the percentage of MSCs in bone

marrow is quite low and decreases with age [25], adipose tissue may become a valuable source of multipotential cells to be used in cell replacement therapy in the future [26, 27].

Genomic studies have provided a more detailed understanding of multipotent stem cells, because the differentiation of stem cells is expected to result in significant changes in gene expression. ASCs express the mesenchymal cell-specific markers and molecular markers typical for the embryonic stem cell phenotype: OCT4, Nanog, and Sox2 [28]. Evaluations of the expression of these genes are used as markers to assess the state of the differentiated cells. The expression of most of them is low in hematopoietic mesenchymal cells. Many of these pluripotency-associated genes have several pseudogenes (genomic DNA sequences similar to normal genes, and regarded as defunct relatives of functional genes), so the results of Real-Time PCR analysis should be verified by protein level analysis [29]. Further studies on the role of the regulatory factors in the differentiation of ASC cultured *in vitro* and *in vivo* are expected to explain the molecular mechanisms and highlight some of the transcription pathways involved in the lineage-specific differentiation of these stem cells.

THE DIFFERENTIATION POTENTIAL OF ADIPOSE-DERIVED STEM/STROMAL CELLS

As mentioned above, two functionally different types of fat are found in humans: WAT and BAT. Stem cells isolated from each type differ in number and differentiation potential. Generally, adult stem cells from WAT exhibit a higher differentiation potential, are more abundant, and grow faster than cells isolated from BAT [30, 31]. There are differences in the ASC population, even when the cells are isolated from different anatomical regions of the same type of adipose tissue [32, 33]. Thus, ASCs are a heterogeneous group of progenitor cells, but this does not affect their great potential for the stem cell field, in particular for tissue engineering. Recent research showed that unsorted ASCs are an efficacious source of multipotent cells, and have the ability to differentiate into several different cell types [34-36].

ASCs are capable of secreting a large number of cytokines and growth factors that support angiogenesis, tissue remodeling, and antiapoptotic events, such as: VEGF, HGF, Il-6, Il-7, TNF α , M-CSF, and TGF- β 1 [37]. Cytokines and growth factors produced by cultured cells (other than those added externally to the culture) might affect cell differentiation, and might control and manage the neighboring cells.

The formation and regeneration of every tissue is associated with a cascade of signals involving a sequential activation of successive genes in response to growth factors and transcription regulators. Optimal culture conditions and proper stimulation can induce the *in vitro* differentiation of multipotent cells into a desirable cell phenotype. The mechanisms that drive the ASCs into the specialized lineage are not clear, and experimental work is required to provide

an understanding of the role and interaction of many factors and signal cascades in the process of cell differentiation and maturation. The proper lineage-specific differentiation is directly related to the expression of key transcription factors of mature tissue. The direction of the cell specialization is estimated on the basis of their type, i.e. the process of bone cell differentiation can be monitored based on the expression of two key transcription factors in osteoblastogenesis: Runx2 and Osterix [38].

Adipogenic differentiation

Adipose tissue transplantation was primarily used for human reconstructive surgery. It is obvious that ASCs have an exceptional potential for differentiation into mature adipocytes [39-41], which is very promising in developing improved techniques to repair soft tissue defects, especially after oncological surgery, e.g. breast reconstruction after a mastectomy [42]. This type of differentiation occurs *in vitro* under the influence of insulin, isobutylmethylxanthine, dexamethasone, rosiglitazone, and indomethacin. The adipocytes obtained by stimulation of ASCs have a specific lipid vacuole in the morphology of the cell, and express several genes and proteins involved in lipid biosynthesis, metabolism and accumulation, including leptin, peroxisome-proliferating activated receptor γ (PPAR γ), glucose transporter type 4 (GLUT4), and glycerol-3-phosphate dehydrogenase (GPDH). Genetic and biochemical analysis confirmed the adipose differentiation of ASCs, as described in the first paper in the field of human adipose-derived stem cells, by Zuk *et al.* [7, 43]. In that study, ASCs obtained from many donors were cultured with appropriate medium supplementation, and they differentiated into not only adipogenic lineages, but also osteogenic, chondrogenic, and myogenic lineages. Thus, ASCs represent a promising strategy for skeletal tissue regeneration.

Osteogenic differentiation

Under osteogenic conditions (medium supplemented with dexamethasone, β -glycerophosphate and vitamin D₃), ASCs are observed to express genes and proteins associated with the osteoblast phenotype, including alkaline phosphatase, type I collagen, osteopontin, osteonectin, and Runx2 [40, 41, 44]. When a medium is supplemented with BMP2, the most osteogenic protein from the group of bone morphogenetic proteins, or there is an enhanced expression of BMP2 in progenitor cells, osteogenic differentiation is stimulated. It was demonstrated *in vitro* and *in vivo* that ASCs could be induced to efficiently differentiate into osteoblasts by the transfection of osteogenic lineage-determining genes, i.e. BMP2 and Runx2 [45]. ASCs undergoing osteogenic stimulation are able to adhere to scaffolds, migrate, proliferate, and differentiate in order to restore the function of bone tissue *in vivo* [46-48]. This kind of scaffold/cell construct can be effective to regenerate damaged bone tissue lost through disease or accident, or absent due to malformation [36, 47, 49-51].

Chondrogenic differentiation

Several *in vitro* studies have shown the chondrogenic differentiation of ASCs using a medium growth factor supplemented with insulin growth factor (IGF), bone morphogenetic proteins (BMPs), and transforming growth factor- β (TGF- β) [52-55]. The chondrogenic differentiation potential of ASCs was confirmed by their ability to generate cartilage in a variety of experimental models. ASCs seeded into polyglycolic acid (PGA) scaffolds under *in vitro* cultured dynamic conditions exhibited chondrogenic characteristics and synthesized cartilage extracellular matrix components within a 2-week period [56]. Interestingly, Betre and colleagues demonstrated that ASCs on elastin-like polypeptide material can grow and express the chondrogenic phenotype without the supplemental factors that cause chondrogenic differentiation [57]. The great potential of ASCs in cartilage tissue engineering was also demonstrated in studies *in vivo*, i.e. during the implantation of stem cells isolated from human adipose tissue in animal models, such as nude mice [58].

Myogenic differentiation

ASCs possess the capacity to differentiate and display a myogenic phenotype *in vitro* [7, 59]. The main factors activating the expression of genes relevant to myogenesis *in vitro* (heavy chain of myosin, myogenic determination factor 1 – MyoD1) are dexamethasone and hydrocortisone in the medium. The expression of these genes is characteristic for satellite cells during embryogenesis. Terminally differentiated myoblasts can form multinucleated myotubules, and have the ability to shrink and diastole under the influence of atropine. *In vivo* studies showed that the implantation of ASCs in X-linked muscular dystrophy mice restored the dystrophin expression in the muscles of mice [60]. These results are particularly promising in the context of human Duchenne muscular dystrophy, a genetic disease characterized by progressive muscle degeneration and weakness.

Numerous studies have demonstrated that ASCs retain the differentiation potential towards the cardiomyogenic lineage in the presence of interleukine (IL-3 and IL-6) in the medium. Cardiomyocyte-like cells generated by the differentiation of ASCs exhibit phenotypes resembling native heart myocytes. What is more, experimental studies indicate that an adequate induction of cardiomyogenic ASC differentiation can be achieved and improved by incubating the stem cells with exogenous nucleosides [61]. Stem cells from adipose tissue might become a preferable cell source for repairing damaged cardiovascular tissues, such as in the ischemia or infarcted heart.

Endothelial differentiation

The regenerated tissues need to contain vascular systems to allow both the tissue and the differentiated cells to survive. Thus, vascularization of regenerated tissues is an important field of research. It has been reported that human ASCs have the potential for endothelial differentiation [27, 34, 62-66] and can participate in blood vessel formation. ASCs are a possible cell source for vessel

regeneration, as they are able to secrete a number of proangiogenic factors, like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) [67, 68]. A particularly interesting target tissue for reconstructive surgery in the context of vascularization seem to be grafts vascularized in a one-step procedure. The first promising studies aiming to develop an osteogenic and vasculogenic construct using human adipose stromal-vascular cell fractions were performed by Martin *et al.* from Basel University Hospital [66, 69]. They demonstrated that human ASCs under perfusion flow in a three-dimensional environment are able to form bone tissue and blood vessels after implantation in nude mice. What is more, the blood vessels formed by human ASCs were functionally connected to the mouse vascular network and contained mouse erythrocytes.

Neuronal differentiation

Many studies have confirmed the neurogenic potential of ASCs. The original Zuk *et al.* article from 2002 suggests that ASCs might possess the ability to form neuronal cells. Adipose tissue-derived stem cells that differentiate into putative neurogenic cells, exhibit a neuronal-like morphology and express several proteins consistent with the neuronal phenotype (Neuron Specific Enolase – NSE; Neuron Specific Nuclear Protein – NeuN) in a medium containing valproic acid, insulin, hydroxyanisole, epidermal growth factor (EGF) and fibroblast growth factor (FGF) [43].

The latest experiments confirm the ability of ASCs to differentiate into cells of epidermal lineage [70, 71]. The ideal situation would thus be to receive a vascularized and innervated tissue for autologous transplantation. Theoretically, ASCs may provide an efficient and convenient autologous source of cells for advanced tissue engineering for clinical therapies.

As the number of patients with diabetes is increasing, insulin-producing cells derived from mesenchymal stem cells provide an attractive alternative treatment for patients who have lost their own cells responsible for this process. ASCs are able to adopt a pancreatic endocrine phenotype *ex vivo* (induction of the insulin, glucagon, and somatostatin genes) in response to defined culture conditions (high glucose concentration, nicotinamide, hepatocyte growth factor, activin-A, pentagastrin) without genetic modification. Thus, ASCs could be used as a model to develop stem cell-based therapies for diabetes mellitus [35, 72, 73].

Few studies have reported an epithelial differentiation of ASCs. A tissue-engineered airway construct with a three-dimensional structure of fibrin and ASCs was created as a prototype vocal fold replacement. The expression of the early cytokeratin in combination with a decreased vimentin expression is considered the first step toward epithelial differentiation. Long *et al.* showed that under optimized conditions, ASCs express epithelial marker proteins and complete their epithelialization after implantation [59, 74].

The transplantation of hepatocytes might become easier, more efficient and safer than whole organ transplantation in treating patients suffering from final-stage

liver dysfunction. The differentiation of ASCs into hepatocyte-like cells has also been investigated [76]. Thus, the generation of hepatocytes derived from ASCs holds considerable promise for future clinical applications. Human ASCs were transplanted into the livers of immunodeficient mice with or without prior hepatocyte differentiation *in vitro*, and it was observed that the pre-differentiation of ASCs *in vitro* promotes the hepatic integration *in vivo* [77].

Tab. 1. Studies into the use of Adipose Stem Cells – selected applications.

Application	Model	Reference
Adipogenic differentiation	Human ASCs <i>in vitro</i>	[19]
	Human ASCs in athymic nude rats	[39]
Chondrogenic differentiation	Human ASCs <i>in vitro</i>	[56, 57, 91, 92]
	Human ASCs in SCID mice	[54]
Osteogenic differentiation	Human ASCs <i>in vitro</i>	[93-96]
	Human ASCs in nude mice	[97]
	Human ASCs in SCID mice	[19, 98]
	Human ASCs in athymic mice	[45]
	Human ASCs in rats	[75]
	Rat ASCs in SD rats	[40]
Myogenic differentiation	Human ASCs <i>in vitro</i>	[60, 99-101]
	Human ASCs in <i>mdx</i> mice	[102]
	Human ASCs in nude mice	[34]
Cardiomyogenic differentiation	Human ASCs <i>in vitro</i>	[103, 104]
	Rat ASCs in rats	[105]
Neuronal differentiation	Human ASCs <i>in vitro</i>	[43, 106]
	Canine ASCs in dogs with spinal cord injuries	[107]
	Rat ASCs in rats	[70]
Osteogenic constructs with intrinsic vascularization	Human ASCs in nude mice	[66, 69]
Epithelial differentiation	Human ASCs <i>in vitro</i>	[59, 74]
Kidney differentiation	Human ASCs in C57BL/6 mice	[108]
Pancreatic differentiation	Human ASCs <i>in vitro</i>	[72, 73]
Hepatocyte differentiation	Human ASCs in Pfp/Rag2 knockout mice	[77]
	Human ASCs in Ccl4 mice	[76]
Periodontal tissue regeneration	Rat ASCs in rats	[109]

The proposed uses for ASCs in tissue engineering and regenerative medicine are collected in Tab. 1. Among them, the Choi group showed the model of regeneration of the intervertebral disc in rats by implanting ASCs. Intervertebral disc degeneration (IVD), one of the causes of lower back pain, is an irreversible process for which no restorative treatments are currently available. The etiology

remains unknown, but the condition can be described clinically as a loss of proper stability and mobility. Human ASCs were transplanted into damaged disc segments in rats, and were found to restore the degenerated intervertebral disc [75]. Based on the promising results of animal model studies, certain clinical trials have also been initiated [78]. There is a growing number of preclinical studies examining the potential of ASCs. The ongoing human clinical trials are summarized in Tab. 2.

Tab. 2. Ongoing ASCs human clinical trials (based on www.clinicaltrials.gov).

Condition	Title	Design
Complex Perianal Fistulas	Efficacy and Safety of Adipose Stem Cells to Treat Complex Perianal Fistulas Not Associated to Crohn's Disease (FATT1)	Phase III
Type 1 Diabetes Mellitus	Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Patients With Type 1 Diabetes	Phase I/II
Lipodystrophy	Autologous Adipose-Derived Stem Cell Transplantation in Patients With Lipodystrophy	Phase I
Chronic Critical Limb Ischemia	Intraarterial Infusion of Autologous Mesenchymal Stem Cells From Adipose Tissue in Diabetic Patients With Chronic Critical Limb Ischemia	Phase I/II
Crohn Disease	Treatment of Fistulous Crohn's Disease by Implant of Autologous Mesenchymal Stem Cells Derived From Adipose Tissue	Phase I/II
Autoimmune Diseases Immune System Diseases Demyelinating Diseases Nervous System Diseases Demyelinating Autoimmune Diseases, CNS Autoimmune Diseases of the Nervous System	Autologous Mesenchymal Stem Cells From Adipose Tissue in Patients With Secondary Progressive Multiple Sclerosis	Phase I/II
Type 2 Diabetes Mellitus	Safety and Efficacy of Autologous Adipose-Derived Stem Cell Transplantation in Type 2 Diabetics	Phase I/II
Rectovaginal Fistula Crohn Disease	Allogenic Stem Cells Derived From Lipoaspirates for the Treatment of Recto-Vaginal Fistulas Associated to Crohn's Disease (ALOREVA)	Phase I/II
Fecal Incontinence	Safety Study of Autologous Cultured Adipose - Derived Stem Cells for the Fecal Incontinence	Phase I
Crohn's Fistula	Safety and Efficacy Study of Autologous Cultured Adipose -Derived Stem Cells for the Crohn's Fistula	Phase I
Myocardial Infarction Coronary Arteriosclerosis Cardiovascular Disease Coronary Disease	Randomized Clinical Trial of Adipose-Derived Stem Cells in the Treatment of Pts With ST-Elevation Myocardial Infarction	Phase I
Complex Perianal Fistula	Long-term Safety and Efficacy of Adipose-derived Stem Cells to Treat Complex Perianal Fistulas in	Phase II

Condition	Title	Design
	Patients Participating in the FATT-1 Randomized Controlled Trial	
Crohn's Fistula	Safety and Efficacy Study of Autologous Cultured Adipose -Derived Stem Cells for the Crohn's Fistula	Phase II
Ischemic Heart Disease Coronary Arteriosclerosis Cardiovascular Disease Coronary Disease Coronary Artery Disease	A Randomized Clinical Trial of Adipose-Derived Stem Cells in Treatment of Non Revascularizable Ischemic Myocardium	Phase I
Leukemia Hodgkin's Lymphoma Non-Hodgkin's Lymphoma Myelodysplastic Syndrome	Abdominal Obesity and Cardiovascular Risk Factors in Women Who Survived Cancer or a Related Illness Following Total Body Irradiation and Stem Cell Transplant	No available information
Anal Fistula	Autologous Stem Cells Derived From Lipoaspirates for the Non-Surgical Treatment of Complex Perianal Fistula	Phase II
Liver Cirrhosis	Liver Regeneration Therapy Using Autologous Adipose Tissue Derived Stromal Cells	No available information
Breast Neoplasms Carcinoma, Ductal, Breast Mammoplasty Mastectomy, Segmental, Lumpectomy, Breast Reconstruction, Liver Cirrhosis	Study of Autologous Fat Enhanced w/ Regenerative Cells Transplanted to Reconstruct Breast Deformities After Lumpectomy	Phase IV
	Liver Regeneration Therapy by Intrahepatic Arterial Administration of Autologous Adipose Tissue Derived Stromal Cells	No available information
Changes in Bone Mineral Content Changes in Bone Marrow Adipose Tissue	Tissue Partitioning in Early Childhood	No available information
Aging	Age-Related Changes in Proliferation and Differentiation Capacity of Human Preadipocytes From Periorbital Fat	Phase I
Depressed Scar	Safety and Efficacy of Autologous Cultured Adipocytes in Patient With Depressed Scar	Phase II/III
Diabetic Wounds Venous Stasis Wounds	The Role of Lipoaspirate Injection in the Treatment of Diabetic Lower Extremity Wounds and Venous Stasis Ulcers	No available information

PERSPECTIVES

Today, the ability of ASCs to form multiple cell types of all three germ layers (muscle and bone of mesodermal lineage; hepatocytes and pancreatic islets of endodermal lineage; and neurons, oligodendrocytes and functional Schwann cells of epidermal lineage) suggests that ASCs may be pluripotent rather than multipotent stem cells [1, 72, 79-82]. This enormous plasticity of ASCs distinguishes these cells from other thus far characterized stem cells.

ASCs have another important advantage over the cell population isolated from postnatal tissue. A breakthrough study has demonstrated the possibility to efficiently reprogram cells to pluripotency. Induced pluripotent stem (iPS) cells are a type of pluripotent stem cell artificially derived from non-pluripotent cells, typically adult somatic cells, by inducing the expression of transcription factors characteristic for undifferentiated embryonic stem cells: Oct4, Sox2, Nanog, and c-MYC [83-85]. In the area of reprogramming somatic cells to a pluripotent state, ASCs might be more useful than other type of cells. Impressively, Sun and his collaborators generated human iPS cells from ASCs in a shorter period and with higher efficiency than in comparable studies targeting adult human fibroblasts [86].

Additionally, mature cells, obtained from ASCs and cells reprogrammed from ASCs, may be used for drug discoveries in disease models. Their potential use in toxicology models might reduce the need for experimental animals. ASCs may offer an efficient tool for cell-based gene therapy approaches, as they may prove to be a good carrier of genes which are important in the healing process. That is because ASCs can easily and efficiently (above 60%) be transduced with vectors, e.g. an encoding proapoptotic ligand (i.e. tumor necrosis factor-related apoptosis-inducing ligand – TRAIL) that induces apoptosis in tumor cells but not normal tissue [87, 88].

Other engineering manipulations on ASCs may unfold the true potential of these stem cells. These achievements may bring new opportunities to explore innovative therapeutic models or targets in regenerative personalized medicine. Future study into the conditions necessary to optimize the differentiation of ASCs into deficit cells may unlock the therapeutic potential of ASCs in regenerative medicine.

The relative abundance and impressive plasticity of human ASCs have raised high enthusiasm over their therapeutic potential. However, recent studies indicate that ASCs are capable of secreting various tumor-promoting factors including IL-6 [89, 90]. Promotion of angiogenesis and vasculogenesis by the ASCs is also discussed in the context of an increased risk of malignant transformation. In addition, the spontaneous conversion of ASC after prolonged incubation *in vitro* also poses a safety hazard in cell transplantation.

Another disadvantage of ASCs is their not completely homogeneous cell population. There is no single phenotype or even a unique antigen to define adipose stem cells. ASC samples isolated from different individuals but also from different anatomical regions of the same patient display significant differences in the inducibility of various secreted factors. The cell population may vary according to age and health.

Many issues must be explored before the safe application of these cells in the clinical setting. However, with appropriate validation of cell types and optimal performance, and further characterization, ASCs should yield a demonstrable benefit in cell therapy.

SUMMARY

ASCs hold great promise for use in tissue repair and regeneration. Among the stem cell-based therapeutic modalities, the use of ASCs seems to be especially valuable in the clinical perspective due to their ready availability, pro-angiogenesis and anti-apoptotic factor secretion, immunomodulatory effects, and the capacity for multi-lineage differentiation. The procedure for isolating ASCs is relatively simple, fast and safe. It is easy to obtain a large quantity of ASCs as a starting population for further usage, e.g. in tissue engineering or reprogramming. ASCs may be isolated from patients at any age, and the proposed uses of these cells in tissue regeneration are truly impressive, making ASCs one of the most popular adult stem cells currently explored. The biology and potential therapeutic applications of adult stem cells is in the scope of current research interest. The successful use of all types of stem cells in regeneration therapy may be achieved only after we learn about their extensive characteristics in detail.

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