VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 26 April 2013
Final form accepted: 09 August 2013
Published online: August 2013

Volume 18 (2013) pp 479-493
DOI: 10.2478/s11658-013-0101-4

Mini review

ADIPOSE TISSUE-DERIVED STEM CELLS SHOW CONSIDERABLE PROMISE FOR REGENERATIVE MEDICINE APPLICATIONS

IZABELA HARASYMIAK-KRZYŻANOWSKA, ALICJA NIEDOJADŁO, JOLANTA KARWAT, LIDIA KOTUŁA, PAULINA GIL-KULIK, MAGDALENA SAWIUK and JANUSZ KOCKI*
Department of Clinical Genetics, Medical University of Lublin, ul. Radziwiłłowska 11, 20-080 Lublin, Poland

Abstract: The stromal-vascular cell fraction (SVF) of adipose tissue can be an abundant source of both multipotent and pluripotent stem cells, known as adipose-derived stem cells or adipose tissue-derived stromal cells (ADSCs). The SVF also contains vascular cells, targeted progenitor cells, and preadipocytes. Stromal cells isolated from adipose tissue express common surface antigens, show the ability to adhere to plastic, and produce forms that resemble fibroblasts. They are characterized by a high proliferation potential and the ability to differentiate into cells of meso-, ecto- and endodermal origin. Although stem cells obtained from an adult organism have smaller capabilities for differentiation in comparison to embryonic and induced pluripotent stem cells (iPSs), the cost of obtaining them is significantly lower. The 40 years of research that mainly focused on the potential of bone marrow stem cells (BMSCs)

* Author for correspondence. e-mail: janusz.kocki@umlub.pl, tel.: +48 81 5288408, fax: +48 81 5288408

Abbreviations used: ADSCs – adipose-derived stem cells or adipose tissue-derived stromal cells; ASCs – adult stem cells; BAT – brown adipose tissue; BMD – Becker muscular dystrophy; BMSCs – bone marrow stem cells; CAL – cell-assisted lipotransfer; CCL5 – chemokine C-C-motif; CD – cluster of differentiation; CFU-F – fibroblast colony-forming unit; DMD – Duchenne muscular dystrophy; DMSO – di-methyl sulfoxide; ESC – embryonic stem cells; FSC – fetal stem cells; HGF – hepatocyte growth factor; HSC – hematopoietic stem cells; Isl 1 – islet 1; iPSs – induced pluripotent stem cells; Kyn – kynurenine; LDLs – low-density lipoproteins; LIF – leukemia inhibitory factor; MHC – major histocompatibility complex; MSC – mesenchymal stem cells; NGN 3 – neurogenin 3; Pax 4 – paired box gene; PDX 1 – pancreatic duodenal homeobox; PGE_2 – prostaglandin E_2; SDF-1 – stromal cell-derived factor; SVF – stromal-vascular cell fraction; TGFβ – transforming growth factor-β; TGFβ1 – transforming growth factor-beta 1; VEGF – vascular endothelial growth factor; WAT – white adipose tissue

revealed a number of negative factors: the painful sampling procedure, frequent complications, and small cell yield. The number of stem cells in adipose tissue is relatively large, and obtaining them is less invasive. Sampling through simple procedures such as liposuction performed under local anesthesia is less painful, ensuring patient comfort. The isolated cells are easily grown in culture, and they retain their properties over many passages. That is why adipose tissue has recently been treated as an attractive alternative source of stem cells. Essential aspects of ADSC biology and their use in regenerative medicine will be analyzed in this article.

Key words: Adipocyte, Mesenchymal stem cells, Regenerative medicine, Adipose tissue, Stem cell therapy, Adipose-derived stem cells, Stromal cells, Flow cytometry

STEM CELLS — DEFINITION AND TYPES

Stem cells are generally defined as poorly differentiated and capable of self-renewal and differentiation into one or more types of specialized cells. Their classification is based mainly on their potential to differentiate into specialized cells, tissues and organs, or even whole organisms [1].

Stem cells are divided into three main groups: embryonic stem cells (ESCs), fetal stem cells (FSCs) and adult stem cells (ASCs). Stem cells of fetal origin include cells of the fetal tissue, umbilical cord blood, placenta and amniotic fluid, and hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) [2]. The following types of adult stem cell can be distinguished: bone marrow stem cells (BMSCs), myosatellite cells, neural stem cells, hematopoietic stem cells (HSCs) and adipose-derived stem cells (ADSCs) [3]. The International Society for Cellular Therapy (ISCT) suggested that cells previously described as mesenchymal stem cells should now be referred to as multipotent mesenchymal stromal cells. According to the ISCT, the term mesenchymal stem cells should be reserved only for subpopulations that show distinct features of stem cells [4].

THE POTENTIAL AND DIRECTIONS OF STEM CELL DIFFERENTIATION

Up to the blastocyst stage, ESCs are totipotent, i.e., able to differentiate into any cell type. Embryoblast cells are pluripotent, which means that they can differentiate into all types of fetal cell apart from placental cells. In the next stages of ontogenetic development, human stem cells lose their pluripotency and become multipotent. It should be emphasized that with time, stem cells' potency and capacity for self-renewal reduces [2]. Thus, ASCs have more limited potential for growth than ESCs. Until recently, it was thought that ASCs are multipotent and differentiate into cells from one and the same germ layer. However, some have been found to only be unipotent, such as myosatellite cells, which transform into one cell type [3, 5].

Due to the limited capacity for differentiation of adult stem cells, new methods for obtaining induced pluripotent cells (iPS) needed to be developed. This was achieved with genetic manipulation that aimed to transform the already differentiated cells into stem cells. The first iPSs were obtained from fibroblasts. Research carried out in recent years showed that under appropriate conditions, ASCs may become pluripotent, and their reprogramming might be easier than reprogramming fibroblasts [6]. Sun *et al.* [7] obtained induced pluripotent stem cells through viral transduction of human ASCs, and realized that this method is faster and more efficient than the induction of pluripotency in human fibroblasts. Subsequent studies confirmed and supplemented these findings with regard to ASCs in mice. The relatively high efficiency of iPS creation from ASCs may be partly due to the high expression of pluripotency factors in ASCs, such as basic-FGF, TGFβ, fibronectin and vitronectin [8].

Obtaining iPSs is methodologically complicated and expensive, so a natural source of pluripotent stem cells is constantly being searched for. Another study showed that ASCs have a greater capability to differentiate into various cell types than originally anticipated. Undoubtedly BMSCs are the best described cell population that exhibits high proliferative potential and the capacity to differentiate into various cell types. Then Zuk *et al.* [9] described the multilineage differentiation of cell populations obtained from enzymatic digestion of human adipose tissue, which led to another breakthrough in the search for a viable stem cell source. In a short time, it was demonstrated that multilineage differentiation was a characteristic of individual cells of this newly isolated population. This observation has been confirmed for both human and mouse ADSCs [10].

ADSCs have great potential for differentiation into cells of mesodermal origin, such as adipocytes, osteoblasts, chondrocytes, myocytes and cardiomyocytes. Studies using *in vitro* conditions demonstrated their high plasticity and ability to differentiate into cells of ectodermal origin (neurons, oligodendrocytes and Schwann cells) and endodermal origin (pancreatic beta cells, hepatocytes and epithelial cells) [3, 4, 11, 12]. The latest reports have extended our knowledge of the differentiation capability of ADSCs to include various types of epithelial cell, such as renal tubular epithelial cells and retinal pigment epithelial cells [8]. Other researchers demonstrated that these cells may have lower potential in bone formation and chondrogenesis than BMSCs, and questioned the importance of adipose tissue as a source of MSCs [13]. In later studies, it was proved that ADSCs can be successfully used in the regeneration of both osseous and cartilage tissues [4].

DILEMMAS IN REGENRATIVE MEDICINE

All groups of stem cells are capable of regenerating damaged tissues and organs. Differences in their development or differentiation potential, self-sufficiency,

and ability to continuously regenerate motivated researchers to use them as a tool in the clinical treatment of various diseases.

ESC have the capacity to proliferate *in vitro* a potentially infinite number of times and toti- or pluripotency, so they could theoretically replace any tissue that has been damaged. However, obtaining these cells destroys the embryo and it has repeatedly been shown that ESCs introduced into an adult body contributed to the formation of teratomas or other types of cancer, instead of integrating and regenerating the tissue. Furthermore, we cannot ignore the problems of immunocompatibility and the ethical issues that effectively prevent the widespread use of this group of cells [2].

Under these circumstances, it is justifiable to focus on adult stem cells, which appear to be more promising in regenerative medicine, including cellular therapy, tissue engineering and even biomedical engineering. It should be noted that adult stem cells are not subject to any ethical considerations. They are immunocompatible and most researchers are convinced that their use is not associated with the process of carcinogenesis or the risk of rejection [2, 5].

ADIPOSE TISSUE AS AN EASILY ACCESSIBLE AND ABUNDANT SOURCE OF STEM CELLS

Adipose tissue covers large areas of an organism and plays an important role in the regulation of its energetic homeostasis. It also has an immunomodulatory role involving the secretion of numerous molecules known as adipokines [5]. In the human body, it exists in two major forms, white adipose tissue (WAT) and brown adipose tissue (BAT). Somatic stem cells of mesenchymal origin have been identified in both types. WAT specializes in energy storage and the production and decomposition of fats, thus acting as the body's metabolic reserve [5, 8].

Adipose tissue is currently the focus of considerable research because it has been recognized that its stromal-vascular cell fraction (SVF) can be an abundant source of multipotent and pluripotent stem cells and adipose-derived stem cells, also called adipose tissue-derived stromal cells (ADSCs) [14, 15]. WAT is a better material for the isolation of ADSCs for several reasons. First, it contains numerous stem cells of high proliferative activity. Second, WAT stem cells demonstrate a higher potential of differentiation than BAT stem cells. They originate in diverse areas, so they are by no means a homogenous population, phenotypically or functionally. That should certainly be considered before their isolation [3].

METHODS OF ISOLATING ADSCs

Recent studies have presented evidence that ADSCs *in situ* are located in the perivascular niche. Unfortunately, standardized techniques for their isolation and cultivation have not yet been developed [12]. ADSCs are mainly extracted from tissue removed during cosmetic liposuction, using a mixture of saline,

epinephrine and lidocaine. Alternatively, adipose tissue may be sampled during surgeries such as hip replacements or the resection of excess adipose tissue.

It was shown that the technique used to obtain the tissue has an impact on the quality of the ADSC population. First of all, to obtain a population of cells of high viability, mechanical treatments that break down the adipose tissue should be limited. These include the use of ultrasound. Ignoring this rule leads to as much as a 70% decrease in the number of viable ADSCs. The sampled material is a mixture of tissue fluid, blood, free fat created as a result of the disintegration of adipocytes, and other tissue fragments that should immediately be submitted to treatment. The test that determines the number of units forming a fibroblast colony (fibroblast colony-forming unit – CFU-F), established that storing samples for 24 h causes a decrease in the number of colonies by up to 50%. Digesting tissue fragments to break them up into single cells is carried out using a mixture of enzymes in the process of extraction, such as collagenase at 35-38ºC. After digestion, the floating fraction is separated from the stromal cells, which have settled on the bottom. On the surface of the centrifuged mixture, there are floating adipocytes, undigested parts of the tissue, and free fat. The stromal fraction (on the bottom) consists of a heterogeneous mixture of cells, containing ADSCs capable of adhesion [10]. The fraction also consists of vascular cells, targeted progenitor cells and preadipocytes [4, 12, 16].

Proper stem cells are isolated from *in vitro* culture. In the first stage of the culture, cells which do not adhere to the plastic are eliminated. In subsequent stages, targeted cells such as progenitor cells and preadipocytes die out. After approximately 8 weeks of growth, only ADSCs remain, i.e., the most primary stem cells, and these can be cultivated *in vitro* in 15 passages or stored at -140ºC [4, 14].

Another assessment of stem cell isolation from adipose tissue showed that there is no need for tissue digestion with collagenase. It was easy to separate the slurry of tissue into adipose and stromal cells during the process of centrifugation. As early as in the first stages of the study, it was shown that the capability of the cells to adhere was higher with this method than with enzyme incubation [17].

Researchers are still looking for more efficient methods of obtaining stem cells from the stromal fraction. Interesting results were published by Wu *et al.* [18]. They demonstrated that human ADSCs may be obtained from a slurry of adipose tissue sampled directly from patients after filtration through a membrane using a polyurethane foam. In this method, there is no need for *in vitro* cell culture and it can be performed in approximately 30 min. Cells isolated using this method showed a high potential for osteogenesis [18].

Thanks to the identification of ADSC surface markers, it is possible to isolate stem cells directly from SVF using flow cytometry and immunogenetic beads used for positive and negative selection [5].

THE PHENOTYPE OF ADSCs

Analysis of the surface markers through flow cytometry showed that MSCs isolated both from bone marrow and adipose tissue have a similar set of surface antigens, including CD105, CD54, CD106 and HLA-1 markers. Furthermore, they are positively stained for the presence of vimentin, ASMA, collagen (collagen-1) and fibronectin, but they do not include HLA-DR, CD117 or hematopoietic cell markers [17].

ADSCs are generally similar but not identical to BMSCs. They show CD105/endoglin, CD44, CD90/Thy 1 and SH2 expression, but do not show CD45 and CD31 expression. However, in different populations of these cells, there were differences in the expression of certain surface molecules, including VCAM-1 and VLA-4; the ability to differentiate into various lines of osseous or cartilage cells; and the basic expression of genes [10]. In 2010, it was shown that ADSCs are STRO-1^+ and $CD34^-$ [19]. BMSCs do not exhibit CD49d expression, while ADSCs do. ADSCs do not exhibit CD106 expression, which is present in BMSCs. Many authors agree on the presence of such markers as CD13, CD29, CD73, CD90, CD133, MHC I and MHC II on the surface of ADSCs. Attention is increasingly drawn to the fact that ADSCs should not be identified on the basis of individual surface markers, but it is advisable to mark their whole panel [4].

Unfortunately, currently available data on ADSC phenotypes are inconsistent and do not allow for a clear identification of the ADSC population in the SVF cell mixture. It should be emphasized that ADSCs have a lot of antigens in common with the other fractions of SVF cells and cannot be totally isolated from the heterogeneous mixture. Among other reasons, this is due to the existence of a number of sub-populations of stem cells in SVF and progressive changes in the phenotype of these cells in *in vitro* cultures [12].

IMMUNOSUPPRESSIVE PROPERTIES OF ADSCs

The immunosuppressive properties of ADSCs have yet to be sufficiently examined. Preliminary studies have shown that they play a role in mitigating symptoms of graft-versus-host-disease (GvHD). They lessen the production of inflammatory cytokines through CD4 T-helper and CD8 Tc1 cells. Furthermore, they stimulate the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) through monocytes and T lymphocytes, which in turn secrete immunosuppressive factors, such as leukemia inhibitory factor (LIF), kynurenine (Kyn) and prostaglandin E_2 (PGE_2). In terms of their functionality, ADSCs are immunologically privileged because they do not exhibit HLA-DR expression (major histocompatibility complex class II – MHC II), and they have the ability to decrease the proliferation of activated lymphocytes [4, 8].

In the future, ADSCs may be used in the treatment of such inflammatory diseases as GvHD, Crohn's disease, sepsis, rheumatoid arthritis and neurodegenerative diseases. Many of these applications of ADSCs are still in

clinical trials, and the mechanism of immunosuppression induced by ADSCs requires further study [8].

REGENERATIVE MEDICINE

Regenerative medicine is an interdisciplinary field of science. It comprises cellular therapy and tissue engineering, in which autologous stem cells play an important role. Cellular therapy is all about the isolation of these cells from the donor tissue, their proliferation and differentiation *in vitro*, and their transplantation into the damaged tissue or organ of the recipient. Tissue engineering is the study of interactions between biomaterials, stem cells and biologically active molecules (e.g. growth factors). The aim is to establish procedures and materials applicable in the regeneration of damaged tissues and organs caused by injuries or as a result of various diseases. Demonstration of the immunomodulatory properties of ADSCs, their differentiation into lines of meso-, ecto- and endodermal origins, and their ability to regenerate various tissues enabled their use as a tool in regenerative medicine.

ADSCs IN LIVER DAMAGE

Some studies have focused on the use of mesenchymal adipose tissue stem cells in regenerating liver tissue. After their isolation and incubation with specific growth factors, such as hepatocyte growth factor (HGF) and fibroblast growth factors (FGF1, FGF4), the $CD105^+$ ADSC fraction showed a strong ability to differentiate into transplantable liver cells, which produced albumin and urea and were able to retrieve low density lipoproteins (LDLs) [11]. In other reports it was shown that apart from HGF, oncostatin and di-methyl sulfoxide (DMSO) induce the hepatocyte phenotype in ADSCs. This is reflected by the expression of not only albumin but also α-fetoprotein. These cells were administered intravenously to mice and showed integration with the liver, leading to the regeneration of the previously removed fragments [20].

The following may be implanted into injured organs: mesenchymal cells after their isolation and proliferation, hepatocytes obtained from mesenchymal cells, and hepatocytes obtained from mesenchymal cells cultured three-dimensionally [2]. There are numerous reasons that the transplantation of hepatocytes may be easier, more effective and safer than the transplantation of a whole organ in patients suffering from advanced liver degeneration [15].

ADSCs AND RECONSTRUCTION OF CARDIAC MUSCLE

Congenital and acquired heart diseases are among the most severe and most common causes of death in the world. Currently, cardiovascular surgery employs different types of bioprosthesis. However, using them is connected with a high risk of complications and there is often a need to replace them if they were implanted during the patient's childhood or adolescence. The existing

treatment strategies, such as angioplasty or by-passes, do not provide long-term regeneration of cardiac vessels and tissues [21]. In recent years, attempts to regenerate cardiac muscle tissue using stem cells, including ADSCs, has evoked great enthusiasm from many researchers and clinicians.

After a myocardial infarction, the tissue is chronically damaged. In the first stage, muscle cells die as a result of ischemia. Then, the area of damaged tissue increases due to apoptotic cell death resulting from the death factors secreted by the necrotic cells. Successful regeneration of the cardiac muscle consists of three basic principles: restoring cardiomyocytes, restoring functionality of the venous network, and returning to the healthy geometry of cardiac ventricles. All these aspects of cardiac tissue regeneration can be supported by ADSC transplantation. It has been proven that ADSCs have the capability to differentiate into cardiomyocytes, vascular smooth muscle cells and endothelial cells, and they demonstrate paracrine activity in the damaged tissue. In practice, it has been shown that a relatively small percentage of transplanted cells populate the area of the damaged tissue, and only a few of them differentiate in the desired direction. It is not sufficient to reproduce the aforementioned area, so it was more important to focus research on the paracrine activity of the transplanted cells [22].

The fact that ADSCs secrete angiogenic factors, such as hepatocyte endothelial growth factor (HGF) and vascular endothelial growth factor (VEGF), shows their pro-angiogenic action, which in turn leads to the formation of blood vessels or neovascularization [8, 16]. These cytokines are growth factors, which are important in the treatment of tissue by affected by ischemia. VEGF and HGF induce capillary formation in the heart. Moreover, HGF decreases apoptosis in cells exposed to the effects of ischemia, mitigates fibrosis of the injured tissue, and reduces ventricular hypertrophy. Not without significance is the fact that ADSCs secrete transforming growth factor-beta 1 (TGFβ1), which regulates the inflammatory phase and functions of fibroblasts in the injured tissue. Another important role of ADSCs is mitigating the inflammatory response. It has been shown that the subpopulation ADSC $CD34^+/CD31^-$ exhibits expression of receptor CXCR-4 for SDF-1 chemokine on its surface. The creation of the chemokine by cells of the damaged tissue attracts ADSC $CD34^+/CD31^-$. Taking into consideration that adipose stem cells also secrete chemokine SDF-1, it contributes to the chemotaxis of other progenitor cells [22].

The influence of ADSCs on damaged cardiac muscle tissue has been extensively studied in animals. ADSCs are transplanted in both the acute phase immediately following the induced myocardial infarction, and during the phase of chronic tissue damage, a few weeks after the incident. In both cases, their positive effects on regeneration and retrieval of cardiac function could be observed. Yamada *et al.* [23] demonstrated that, after their transplantation to the area affected by myocardial infarction, isolated brown adipose tissue cells differentiate in mice into cardiomyocytes and endothelial cells of blood vessels. Improvement in the functioning of ventricles and reduction in the damaged area

were observed. Similar results were discovered in induced myocardial infarction in pigs. Applying autologous ADSCs into the coronary vessels led to a major improvement in the functioning of the left ventricle in the affected area and led to an increase in the number of blood vessels in the regenerated tissue over the course of one month [24].
The results of the research carried out on animals give hope for the possibility of using ADSCs in the near future in the treatment of heart diseases in humans. In the US, advanced clinical trials are ongoing. In these trials, autologous ADSCs are used for the regeneration of the human heart in patients after myocardial infarction. A special apparatus samples adipose tissue from a patient. After processing and isolating the stem cells, the apparatus injects them into the heart in real time [2].

ADSCs IN THE REGENERATION OF NERVOUS TISSUE

The nervous system has a very limited regenerative capacity. Mature neurons do not divide, and stem cells from nervous tissue have a limited potential for forming functional neurons in response to an injury. Wang *et al.* [25] showed that ADSCs are able to differentiate into neurons in a three-dimensional *in vitro* culture with nerve cells. These results gave rise to hopes about the use of ADSCs in the regeneration of the central nervous system, including in cases of neurodegenerative diseases, strokes or spinal cord injuries. As in the case of cardiac muscle repair, the pro-angiogenic activity of ADSCs may accelerate recovery from brain hemorrhage or support the treatment of stroke [8].
The results of *in vivo* studies with animal models have shown that ADSCs transplanted into the brain secrete numerous growth factors and cytokines that are neuroprotective, but do not differentiate into nerve or glial cells. Such results were obtained in a rat model of intracerebral hemorrhage and a cerebral stroke model. Transplanted ADSCs reduced apoptosis and inflammation, decreased loss of neural tissue and had a positive effect on the general condition of animals, despite the fact that they differentiated mainly in the cells of the endothelial line. ADSC differentiation into nerve cells was obtained in a rat model of cerebral cortex ischemia (through middle cerebral artery occlusion) after the application of azacitidine (a DNA methylation inhibitor). In these experiments, ADSCs were administered into the lateral ventricle of brain, and were discovered to migrate to various areas of brain, accumulating mainly in the parts of the damaged cerebral cortex. Application of these cells resulted in improved of motor functions and reduced functional deficits in tested animals. Furthermore, the tests showed that ADSCs may be genetically engineered and used as efficient vectors for gene transfer and brain tissue expression [24].
In another study, an autologous ADSC transplant was used in Niemann-Pick disease (lysosomal storage disorder), the symptoms of which manifest in a variety of organs, including in the cerebellum, where neurodegeneration is observed. ADSCs were isolated from sick mouse adipose tissue, cultured *in vitro*

and then transplanted directly into the affected mice brains. The results showed that adipose tissue stem cells increased the survival of endangered Purkinje cells in the cerebellum, reduced the symptoms of inflammation, and restored the motor coordination of animals. These results are hopeful as regards the use of ADSCs in the treatment of neurodegenerative diseases [26].

ADSCs AND THE IMPROVEMENT OF SKELETAL MUSCLE FUNCTION

Until recently, treating patients with Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) was limited to prenatal diagnosis and genetic counseling or to symptomatic treatment. A more profound understanding of the molecular pathogenesis of muscular dystrophy gives new possibilities for improving the therapeutic procedures.

Vieira *et al.* [27] demonstrated that, after application of dexamethasone, hydrocortisone, and fetal bovine and horse serum, human ADSCs in *in vitro* culture differentiated into myotubules demonstrating dystrophin expression after approximately 45 days. Afterwards, co-cultures were grown to see whether ADSCs would fuse with myoblasts and myotubules. Two types of co-culture were grown. In the first, human myoblasts and ADSCs were cultured in equal proportions, and in the second, DMD myotubules and regular ADSCs were cultured in the proportion 3:1. After using the nutrient medium, which stimulated cell fusion, it was found that myoblasts and ADSCs joined and formed multinucleated myotubules of dystrophin expression. Similarly, DMD myotubules underwent a fusion with regular ADSCs and formed syncytia demonstrating dystrophin expression at the level of regular muscular fibers [24].

ADSCs IN BONE REGENERATION

Bony defects in a patient may be repaired with tissue sampled from another, less important area, but side effects may occur in the areas of such a biopsy. Attempts at bone regeneration with ADSCs have already entered clinical trials. Many scientists are making an effort to use ADSCs differentiated into osteoblasts in anomalous bone unions from accidents, or in cases of bone non-union, ADSCs may support bone tissue transplantation [5]. It has already been shown that under *in vitro* conditions, osteogenesis is supported by adding dexamethasone, ascorbic acid, bone morphogenetic protein 2 (BMP2), vitamin D and beta-glycerophosphate to the medium [28]. Tests have shown that the human and mouse ADSCs that differentiate into osteoblasts participate in the mineralization of extracellular matrix, respond to mechanical stress, and produce alkaline phosphatase and osteocalcin. In subsequent stages, they acquire the characteristics of osteogenic cells. All of the above proves that differentiation into bone cells is sensitive to mechanical stress. This is a good starting point in using ADSCs in tissue engineering [20]. Yang *et al.* [19] also showed that differentiation of ADSCs (phenotype STRO-1, $CD90^+$, $CD44^+$, $CD34^-$) to

osteogenesis is stimulated by a cyclic tensile stretch in the signaling pathway BMP-2 [19].
Lindroos *et al.* [4] described the case of reconstruction of the damaged skull bone with autologous SVF cells immobilized in fibrin gel. As early as three months after surgery, there was progressive reconstruction of new fragments of bone tissue. In another case, before transplantation, autologous ADSCs were combined with a synthetic bone substitute containing rhBMP-2, βTCP and calcium phosphate. After implantation, creation of mature bone structure elements was observed [4].

ADSCs IN DIABETES

Diabetes is a destructive disease characterized by a complete lack of insulin due to the destruction of β cells (type 1). It may also be caused by a relative lack of insulin associated with reduced sensitivity to insulin (type 2). It seems that pancreatic islet cell transplantation may be a potential therapy for patients suffering from diabetes type 1, and it can restore the regular blood sugar level. One of the major problems is the lack of pancreas donors and the high risk of rejection. Autologous ADCSs may eliminate these limitations and become an alternative treatment.
Under *in vitro* conditions, Okura *et al* [29] obtained an effective process of differentiation of ADSCs into pancreatic β cells. According to the published results, stem cells from both bone marrow and subcutaneous adipose tissue have the ability to differentiate in this manner. This is a promising direction of investigation toward more effective treatment of diabetes. Other researchers [30] also discovered that ADSCs differentiate into endocrine cells expressing insulin. ADSCs do that under the influence of high glucose levels in the base (25 mmol/l) and nicotinic acid (Nicotinamide), exendin-4, and 2-mercaptoethanol.
In a recent study, it was found that ADSCs differentiate in complexes like those of pancreatic islets, which are characterized by positive dithizone staining and gene expression for Nestin, pancreatic duodenal homeobox (PDX 1), islet 1 (Isl 1), neurogenin 3 (NGN 3), pai red box gene (Pax 4) and insulin. Moreover, a positive result was obtained in the glucose challenge assay test at different concentrations of glucose, which means that a greater amount of insulin is secreted at higher glucose concentrations. A comparative analysis of ADSCs and BMSCs showed that the latter are more effective when it comes to differentiation into pancreatic islets, and therefore seem to be a better choice for the treatment of this organ [31].

ADIPOSE TISSUE REGENERATION USING ADSCs

Both cellular therapy methods and tissue engineering are used in adipose tissue regeneration with ADSCs. In one method, often referred to as CAL (cell-assisted lipotransfer), sampled autologous adipose tissue is divided in two parts after

obtaining SVF. From the first part, ADSCs are isolated and combined with the second part of lipoaspirate to obtain an adipose tissue substitute enriched with stem cells. This method is very effective for regenerating fragments of adipose tissue. This procedure is already at the stage of clinical trials that compare the long-term results of a traditional autologous transplant with one which is enriched in ADSCs (CAL). The method of lipoaspirate enriched with ADSCs is also used in treating the side effects of radiotherapy. The improved functioning of a patient's tissues is probably caused by the reconstruction of blood vessels [32].
In cases of large tissue loss or breast reconstruction, there have been attempts at their reconstruction using tissue engineering techniques with ADSCs. In this strategy, natural or artificial tissue scaffolds are used. They fill in defects in soft tissue and provide mechanical support for the reconstruction of adipose tissue. It is believed that the biomaterials should be characterized by biocompatibility, degradability, mechanical characteristics similar to those of the soft tissues, and stimulation of reconstruction of the venous network. Natural biomaterials include collagen gels, foams and beads that allow the proliferation and differentiation of *ex vivo* cells, so that they could be injected into the damaged tissue. Collagen type I is used as a scaffold for cell growth or in the form of beads as a carrier that maintains its capacity over a long period of time after its administration to the organism. After approximately 8 weeks of topical administration of such complexes, regeneration of adipose tissue was observed. The formation of capillaries was noted in the transplanted material. Cytokines secreted by ADSCs promoted their growth. There are also trials carried out using synthetic biomaterials such as polyglycolic acid gels [32].

ADSCs IN CANCER THERAPY

Lately, scientific journals have been reporting that after topical and intravenous application, mouse ADSCs gathered in the growing neoplasm and promoted its uncontrolled *in vivo* growth [33]. Stimulation of carcinogenesis may be caused by factors secreted by ADSCs, such as stromal cell-derived factor (SDF-1), chemokine C-C-motif (CCL5) and transforming growth factor-β (TGFβ) [8]. By contrast, Cousin *et al.* [34] showed that ADSCs strongly impede the development of pancreatic cancer *in vivo* and *in vitro* by affecting the cell cycle of these proliferating tumor cells. Other researchers, such as Kucerova *et al.* [35], provided initial evidence in favor of ADSCs, which in the future could be the proper vector in gene therapy of cancer [4].

PROSPECTS FOR THE FUTURE

Some therapies employing stem cells have already entered the stage of clinical trials. They concern patients suffering from diseases such as diabetes types 1 and 2, including chronic limb ischemia; myocardial infarction; cirrhosis of the liver; multiple sclerosis; and fistulas associated with Crohn's disease or other types; and patients who are post-mastectomy or have hard-to-heal wounds [4]. It seems

that ADSCs may also be able to play an important role in treatment of strokes, Parkinson's disease, Alzheimer's disease and retinopathy, and in artificial organ creation and the reconstruction of damaged organs [2]. However, there is still a need to clarify the procedures and specifications and to standardize the methods used in obtaining, growing and using these cells. There are no legal protocols or regulations enabling their sampling, *in vitro* differentiation into selected tissues or organs, and transplantation to the recipient organism. Although a lot of laboratory and clinical research has to be done, stem cells remain extremely promising for regenerative medicine.

Acknowledgements. This study was supported in part by funds from the project "The equipment of innovative laboratories doing research on new medicines used in the therapy of civilization and neoplastic diseases" within the Operational Program Development of Eastern Poland 2007-2013, Priority Axis I Modern Economy, Operations I.3 Innovation Promotion.

REFERENCES

1. Bajek, A., Olkowska, J. and Drewa, T. Mesenchymal stem cells as a therapeutic tool in tissue and organ regeneration. **Postepy Hig. Med. Dosw.** 65 (2011) 124-132.
2. Banaś, A. Stem cells – perspectives and dangers. **Prz. Med. Uniw. Rzesz.** 8 (2010) 117-127.
3. Skalska, U. and Kontny, E. Regenerative and immunomodulatory properties of adipose-derived mesenchymal stem cells. **Post. Biol. Kom.** 38 (2011) 363-378.
4. Lindroos, B., Suuronen, R. and Miettinen, S. The Potential of adipose stem cells in regenerative medicine. **Stem Cell Rev.** 7 (2011) 269-291.
5. Jezierska-Woźniak, K., Nosarzewska, D., Tutas, A., Mikołajczyk, A., Okliński, M. and Jurkowski, M.K. Use of adipose tissue as a source of mesenchymal stem cells. **Postepy Hig. Med. Dosw.** 64 (2010) 326-332.
6. Zuk, P.A. The Adipose-derived stem cell: looking back and looking ahead. **Mol. Biol. Cell** 21 (2010) 1783-1787.
7. Sun, L., Akiyama, K., Zhang, H., Yamaza, T., Hou, Y., Zhao, S., Xu, T., Le, A. and Shi, S. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. **Stem Cells** 27 (2009) 1421-1432.
8. Cawthorn, W.P., Scheller, E.L. and MacDougald, O.A. Adipose tissue stem cells: the great WAT hope. **Trends Endocrinol. Metab.** 23 (2012) 270-277.
9. Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J., Benhaim, P., Lorenz, H.P. and Hedrick, M.H. Multilineage cells from human adipose tissue: implications for cell-based therapies. **Tissue Eng.** 7 (2001) 211-228.

10. Fraser, J.K., Zhu, M., Wulur, I. and Alfonso, Z. Adipose-derived stem cells. **Methods Mol. Biol.** 449 (2008) 59-67.
11. Banaś, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Quinn, G., Okochi, H. and Ochiya, T. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. **Hepatology** 46 (2007) 219-228.
12. Baer, P.C. and Geiger, H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. **Stem Cells Int.** Article ID 812693, (2012). DOI: 10.1155/2012/812693.
13. Im, G.-I., Shin, Y.-W. and Lee, K.-B. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? **Osteoarthr. Cartil.** 13 (2005) 845-853.
14. Schaffler, A. and Buchler C. Concise review: adipose tissue-derived stromal cells-basic and clinical implications for novel cell-based therapies. **Stem Cells** 25 (2007) 818-827.
15. Witkowska-Zimny, M. and Walenko, K. Stem cells from adipose tissue. **Cell. Mol. Biol. Lett.** 16 (2011) 236-257.
16. Olkowska-Truchanowicz, J. Isolation and characterization of adipose tissue-derived stem cells. **Post. Biol. Kom.** 35 (2008) 517-526.
17. Romanov, Y.A., Darevskaya, A.N., Merzlikina, N.V. and Buravkova, L.B. Mesenchymal stem cells from human bone marrow and adipose tissue: isolation, characterization and differentiation potentialities. **Bull. Exp. Biol. Med.** 3 (2005) 138-143.
18. Wu, C.-H., Lee, F.-K., Kumar, S.S., Ling, Q.-D., Chang, Y., Chang, Y., Wang, H.-Ch., Chen, H., Chen, D.-Ch., Hsu, S.-T. and Higuchi, A. The isolation and differentiation of human adipose-derived stem cells using membrane filtration. **Biomaterials** 33 (2012) 8228-8239.
19. Yang, X., Gong, P., Lin, Y., Zhang, L., Li, X., Yuan, Q., Tan, Z., Wang, Y., Man, Y. and Tang, H. Cyclic tensile stretch modulates osteogenic differentiation of adipose-derived stem cells via the BMP-2 pathway. **Arch. Med. Sci.** 6 (2010) 152-159.
20. Olkowska-Truchanowicz, J. Differentiation of adipose tissue-derived stem cells- novel possibilities for tissue engineering and cell-based therapy. **Post. Biol. Kom.** 36 (2009) 217-231.
21. Palpant, N.J. and Metzger, J.M. Aesthetic cardiology: adipose-derived stem cells for myocardial repair. **Curr. Stem Cell Res. Ther.** 5 (2010) 145-152.
22. Mazo, M., Gavira, J.J., Pelacho, B. and Prosper, F. Adipose-derived stem cells for myocardial infarction. **J. Cardiovasc. Transl. Res.** 4 (2011) 145-153.
23. Yamada, Y., Wang, X.-D., Yokayama, S., Fukuda, N. and Takakura, N. Cardiac progenitor cells in brown adipose tissue repaired damaged myocardium. **Biochem. Biophys. Res. Commun.** 342 (2006) 662-670.
24. Villiers, J.A., Houreld, N. and Abrahamse, H. Adipose derived stem cells and smooth muscle cells: implications for regenerative medicine. **Stem Cell Rev.** 5 (2009) 256-265.

25. Wang, B., Han, J., Gao, Y., Xiao, Z., Chen, B. and Wang, X. The differentiation of rat adipose-derived stem cells into OEC-like cells on collagen scaffolds by co-culturing with OECs. **Neurosci. Lett.** 421 (2007) 191-196.
26. Bae, J., Carter, J.E. and Jin, H.K. Adipose tissue-derived stem cells rescue Purkinje neurons and alleviate inflammatory responses in Niemann-Pick disease type C mice. **Cell Tissue Res.** 340 (2010) 357-369.
27. Vieira, N.M., Brandalise, V., Zucconi, E., Jazedje, T., Secco, M., Nunes, V.A., Strauss, B.E., Vainzof, M. and Zatz, M. Human multipotent adipose-derived stem cells restore dystrophin expression of Duchenne skeletal-muscle cells in vitro. **Biol. Cell** 100 (2008) 231-241.
28. Gimble, J.M., Katz, A.J. and Bunnell, B.A. Adipose-derived stem cells for regenerative medicine. **Circ. Res.** 100 (2007) 1249-1260.
29. Okura, H., Komoda, H., Fumimoto, Y., Lee, C.M., Nishida, T., Sawa, Y. and Matsuyama, A. Transdifferentiation of human adipose tissue-derived stromal cells into insulin-producing clusters. **J. Artif. Organs.** 12 (2009) 123-130.
30. Silva, A.C., Percegona, L.S., Franca, A.L., dos Santos, T.M., Perini, C.C., Gonzalez, P., Rebelatto, C.L.K., Camara, N.O.S. and Aita, C.A.M. Expression of pancreatic endocrine markers by mesenchymal stem cells from human adipose tissue. **Transplant. Proc.** 44 (2012) 2495-2496.
31. Marappagounder, D., Somasundaram, I., Dorairaj, S. and Sankaran R.J. Differentiation of mesenchymal stem cells derived from human bone marrow and subcutaneous adipose tissue into pancreatic islet-like clusters *in vitro*. **Cell. Mol. Biol. Lett.** 18 (2013) 75-88.
32. Sterodimas, A., de Faria, J., Nicaretta, B. and Pitanguy, I. Tissue engineering with adipose-derived stem cells (ADSCs): Current and future applications. **J. Plast. Reconstr. Aesthet. Surg.** 63 (2010) 1886-1892.
33. Muehlberg, F.L., Song, Y.-H., Krohn, A., Pinilla, S.P., Droll, L.H., Leng, X., Seidensticker, M., Ricke, J., Altman, A.M., Devarajan, E., Liu, W., Arlinghaus, R.B. and Alt, E.U. Tissue-resident stem cells promote breast cancer growth and metastasis. **Carcinogenesis** 30 (2009) 589-597.
34. Cousin, B., Ravet, E., Poglio, S., de Toni, F., Bertuzzi, M., Lulka, H., Touil, I., André, M., Grolleau, J.L., Péron, J.M., Chavoin, J.P., Bourin, P., Pénicaud, L., Casteilla, L., Buscail, L., and Cordelier, P. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. **Plos One** 7 (2009) e6278. DOI:10.1371/journal.pone.0006278.
35. Kucerova, L., Altanerova, V., Matuskova, M., Tyciakova, S., and Altaner, C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. **Cancer Res.** 67 (2007) 6304-6313.