REVIEW

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State of the art in human adipose stem cells and their role in therapy

ALINA SIMONA ŞOVREA[1)], ADINA BIANCA BOŞCA[1)], ANNE-MARIE CONSTANTIN[1)], ELEONORA DRONCA[2)], ARANKA ILEA[3)]

[1)]*Department of Morphological Sciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2)]*Department of Molecular Sciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[3)]*Department of Oral Rehabilitation, Oral Health and Dental Office Management, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

Abstract

Nowadays, adipose tissue appears to be the most valuable source in regenerative cell therapy, due to the following characteristics: high accessibility, high expression in a large number of individuals, high self-renewal and ability to differentiate, and hematopoietic support to the implant area. Its therapeutic potential has been experimentally observed in a broad spectrum of diseases with high population impact: diabetes, myocardial infarction, Parkinson disease, bone fractures, facial reconstruction or loss of subcutaneous tissue due to congenital abnormalities (*e.g.*, hemifacial microsomy), trauma, burns, and tumors. Over 130 clinical trials using adipose-derived stem cells (ASCs), majority phase I or phase II, have been registered with the *National Institutes of Health* (NIH), and in the short term no adverse reactions or significant risks were identified. Parallel with regulatory frameworks that control their safety and assess their efficacy, phase III trials are being developed. Although transplantation with adipose tissue is becoming more and more popular, there are still important drawbacks and technical challenges to be addressed, and clinical strategies to be developed. This review explores in a concise manner the present body of knowledge concerning ASCs and their implication in therapy.

Keywords: stem cell, mesenchymal, autologous transplantation, stromal vascular fraction, adipose stem cells, therapy.

Introduction

Regenerative medicine is gaining more and more attention as a modern tool for repairing damaged tissues by stimulating body regenerative capacity [1]. Several medical fields (clinical medicine, surgery, molecular biology, genetics, and biotechnologies) provide information and knowledge for this new medical branch. Regenerative medicine relies on different techniques for the harvest, culture, cells and tissues engineering, replacement and regeneration of cells/tissues/organs [1].

For this purpose, different types of cells were over time investigated, including the adult mesenchymal stem cells (MSCs), which are multipotent and can differentiate into all three germinal layers: mesenchymal lines (chondrocytes, osteoblasts, adipocytes, or myocytes) [1], endodermal lines (pancreatic cells and hepatocytes) and ectodermal lines (neurogenic and epithelial cells) [2, 3].

MSCs, frequently isolated from bone marrow, have quickly become the golden standard source of stem cells (due to their wide availability and lack of ethical challenges by comparison with embryonic stem cells) [1]. Due to the decrease in number with age, the proliferation capacity and the highly invasive procedure required for the bone marrow-derived stem cells (BMSCs) harvesting, other sources of MSCs were needed and researched [1].

As a response, at the beginning of the 21st century, an alternative source of MSCs was introduced by Zuk *et al.* [3]: the adipose-derived stem cells (ASCs), morphologically and phenotypically similar to MSCs [1, 4–6]. Due to their location, this new type of cells offers very important advantages over BMSCs: subcutaneous adipose tissue, present in a large amount in human body, is highly accessible and it can be easily harvested with minimal side effects [1, 4–9]; moreover, it is inexpensive, biocompatible [4], grows under standard culture conditions [8] and literature data confirms that it has the highest percentage of stem cells in the body (5000 ASCs/g of fat compared to 1000 stem cells/g of bone marrow) [4, 8]. When compared to BMSCs, ASCs have similar differentiation and immunomodulatory potentials but in long-term cultures, they are genetically and morphologically more stable, with higher proliferation capacities and lower senescence, regardless of patients age [1]. However, while ASCs have a better collagen production, BMSCs have a better osteogenic capacity [1, 10].

ASCs is today the standard terminology used by the stem cells research community, following the proposal of the *International Fat Applied Technology Society* (IFAT) in 2004 [11]. Before that, various other names were given to these cells (Table 1) [10–13].

Table 1 – *Abbreviations and nomenclature of ASCs prior to IFAT consensus*

PLAs	Processed lipoaspirate cells
ADASs	Adipose-derived adult stem cells
AD-MSCs	Adipose-derived mesenchymal stem cells
AMSCs	Adipose mesenchymal stem cells
ASSCs	Adipose stromal stem cells
ADSCs	Adipose-derived stromal cells

ASCs: Adipose-derived stem cells; IFAT: International Fat Applied Technology Society.

The increasing global interest in the use of adipose tissue for regenerative medicine applications is reflected

in the *PubMed* literature and *National Institutes of Health* (NIH) clinical trials findings that have been in a continuous grow since 2001 (Figure 1). The boost in peer-reviewed articles has inflicted in the medical community the idea that adipose tissue is unmatched as the new golden standard source of stem cells; besides the great commercial interest, the subject also created clinical infrastructure opportunities for a growing commercial market [14].

History of adipose grafting techniques

By analyzing the historical evolution of different adipose grafting techniques (from simple fat transfer between different zones, to complicated transplantation strategies [7]), one can see the dramatic changes and significant improvements gained in the current harvesting, processing and implantation methods of lipoaspirates [4, 7]; due to its softness, malleability and regenerative potential [7, 9]), adipose tissue is an excellent filler for remodeling volume body defects and represents a promising approach for regenerative therapies in various clinical applications [10] (Table 2).

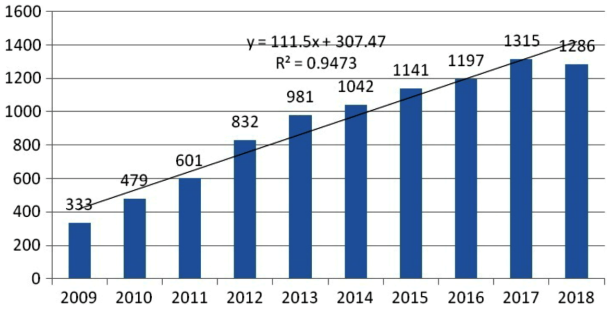

Figure 1 – ***Evolution of trendline in PubMed searching "adipose-derived" AND "stem cells" during last 10 years.***

Table 2 – ***Time points in fat grafting and development of clinical applications***

Century	Performer, year	Technique	Clinical use/finding of adipose tissue	Reference
19th	Van der Meulen, 1889	Autologous fat grafting	To treat a diaphragmatic hernia	[15]
	Neuber, 1893	True adipose graft	To fill a scar on the face	[4]
	Czerny, 1895	Autologous lipoma grafting	In the post-mastectomy reconstruction	[16]
20th	Lexer, 1910	Aesthetic surgery	As filler for the malar infraorbital area	[17]
	Bruning, 1911 & 1914	Fat grafting using a syringe	In aesthetic rhinoplasty, soft tissue augmentation	[18, 19]
	Rehn, 1912	Inject autologous fat	To fill soft tissue defects	[19]
	Hollander, 1912	Fat infiltration	In lipoatrophy of the face	[20, 21]
	Bartlett, 1917	Fat grafting with 50% more than volume required	In breast reconstruction	[22]
	Miller, 1926	Infiltration of fat tissue through cannulas	For the correction of scar contraction on the face	[4, 20]
	Lexer, 1931	Axillar region fat harvesting	In breast reconstruction	[23]
	Peer, 1950 & 1956	Free fat grafts	After one year or more after transplantation adipocytes survival is found to be around 50%; fat grafts lost 45% of mass; mechanical trauma has a negative influence on volume retention	[20, 21, 24]
	Sawhney, 1969	Skin adipose grafts	Discovery of factors which influence graft resorption	[23]
	Fischer, 1974	Developing cannulae with a cutting blade and later, with a blunt hollow	Beginning of the modern liposuction	[25]
	80's–90's	Fat grafting	Establishing surgical parameters for safe use Long-term results for body contour	[26]
	Illouz, 1983	Suction-assisted lipectomy through small cannulas	Increased availability of autologous fat for grafting	[27]
	Fournier & Otteni, 1983	Initiation of "dry" technique: no fluids are injected before liposuction	To promote the benefits of tumescent anesthesia	[28]
	Illouz, 1984	Initiation of "wet" technique: a hypotonic solution (vasoconstrictor and hyaluronidase) is infiltrated before liposuction	To leave intact the blood vessels, the lymphatics, and the nerve endings, found between skin and adipose tissue	[25, 29]
	Fournier, 1985	Microlipoextraction Microlipoinjection	The removed adipose tissue may be used in other locations	[30]
	Klein, 1985	Tumescent technique	The development of lipofilling techniques	[7]
	Coleman, 1987–1998	Manual lipoaspiration under low pressure, centrifugation and reinjection	Decreasing the traumatic mechanical damage of lipoaspiration and centrifugation Reinjecting the cells in direct contact with well-vascularized tissues Improving procedure	[7, 31]
	Fournier, 1989	Injection of non-purified fat	First using of terms: liposculpture or lipofilling	[23]
	Ersek, 1991	Autologous fat microtransplants (blunt cannula with minimal vacuum)	Fat loss between 20–90%	[32]
	Carpaneda & Ribeiro, 1994	Adipose autotransplants	% of fat graft absorption is inversely correlated with the graft thickness	[33]
	Coleman, 1997	Standardized protocol for the processing and placement of lipoaspirate	In aesthetic and reconstructive lipostructure and liposculpting	[31]

Century	Performer, year	Technique	Clinical use/finding of adipose tissue	Reference
21th	Pu *et al.*, 2004 & 2005	Different laboratory assays investigating the viability of the grafted fat	The results favored the Coleman technique (obtaining viable adipocytes in a greater number and more optimal function)	[34, 35]
	Coleman, 2006	Fat grafting through a blunt cannula	In facial rejuvenation and adjustment of proportions Efficacy and permanence of grafted fat are dependent on the harvesting and grafting technique	[36]
	Yang & Lee, 2011	Developing Lipokit™ system, totally enclosed (harvesting, processing, final delivery of the processed fat)	In autologous fat grafting In aesthetic surgery	[37]

ASCs characteristics

ASCs are adult stem cells isolated from adipose tissue [2, 4–6, 8, 9, 11, 38, 39]. In view of *International Society for Cellular Therapy* (ISCT), the ASCs standard definition encompasses certain properties that altogether give the unicity of this cellular type *in vitro* and *in vivo*: plasticity, adherency, undifferentiated state maintenance, proliferation capability with asymmetric division, self-renewal ability, multipotency, multilineal differentiation (including transformation into differentiated functional mature adipocytes) [12, 13, 40].

Still, there are no definitive markers for ASCs, or a personal signature that can specifically define these cells [13]. Generally, it is considered that ASCs are positive for the following cluster of differentiation (CD) antigens: CD_{13}, CD_{29}, CD_{34}, CD_{44}, CD_{49d}, CD_{73}, CD_{90}, CD_{105}, CD_{166}, and negative for the majority of hematopoietic antigens (CD_{11b}, CD_{14}, CD_{19}, and $CD_{79\alpha}$) (Table 3) [12]. Despite the similarities (90%) between ASCs and BMSCs (CD_{29}, CD_{44}, CD_{90}) (Table 3), additional research showed different expression of cell surface markers. For example, CD_{49d} characterizes the ASCs but it is absent in BMSCs, while CD_{49f}, CD_{104}, CD_{106} characterize the BMSCs and are absent in ASCs [10, 12]. The extreme heterogeneity of ASCs profiles (expressing many types of proteins, enzymes, adhesion molecules and receptors), observed by different researchers, may be partly due to donor differences, reagents features (quality, sources, ownership), cell culture conditions, sensitivity of detection or quality of isolation techniques [5, 10, 12].

Table 3 – ***ASCs surface markers***

	ASCs	BMSCs
Positive expression	CD_{10}, CD_{13}, CD_{29}, CD_{34}, CD_{36}, CD_{44}, CD_{49d}, CD_{73}, CD_{90}, CD_{105}, CD_{166}, MHC class I (HLA-A, -B, -C)	CD_{13}, CD_{29}, CD_{44}, CD_{73}, CD_{90}, CD_{49f}, CD_{104}, CD_{106}, CD_{166}, MHC class I (HLA-A, -B, -C)
Negative expression	CD_{11b}, CD_{14}, CD_{19}, CD_{31}, CD_{40}, CD_{40L} (CD_{154}), CD_{45}, $CD_{79\alpha}$, CD_{80}, CD_{86}, CD_{106}, MHC class II (HLA-DR, -DP, -DQ)	CD_{34}, CD_{38}, CD_{40}, CD_{40L} (CD_{154}), CD_{45}, CD_{80}, CD_{86}, MHC class II (HLA-DR, -DP, -DQ)

ASCs: Adipose-derived stem cells; BMSCs: Bone marrow-derived stem cells; CD: Cluster of differentiation; MHC: Major histocompatibility complex; HLA: Human leukocyte antigen.

New data refined the profile of ASCs, indicating a more restrictive but mandatory molecular phenotype, namely: CD_{34-}, CD_{31-}/CD_{45-}, human leukocyte antigen (HLA)-DR- [13].

According to several studies [8, 12, 41], ASCs express CD_{34+} (early progenitor marker) and have a low expression of CD_{105} antigen, profile that does not reflect a proper ISCT BMSC class definition (CD_{34-} and CD_{105+}). The debate was only recently elucidated, since IFAT and ISCT agreed on this regard [41]; a dynamic evolution of both markers was demonstrated [41]. A CD_{34+}/$CD_{105\ low}$ profile characterizes both the ASCs found in fresh, uncultured stromal vascular fraction (SVF) [41] and those from the cultured early passages [8]. After adherent-cell purification of SVF, the ASCs CD_{34+} expression is lost but CD_{105+} expression is gained [41], explaining the different cells profiles. In culture, the expression of CD_{34+} can be maintained for 20 weeks [8], and literature data indicates a greater proliferative capacity for these CD_{34+} ASCs compared with the more plastic CD_{34-} ASCs [8, 10].

Other reported surface markers for a specific ASCs profile are: the protein preadipocyte factor-1 (pref-1) and the pericytic markers {*e.g.*, the platelet-derived growth factor receptor beta (PDGFR-β) and 3G5, or the associated presence of $\alpha 5\beta 1$ integrin [42]}.

The understanding and standardization of ASCs surface phenotype are very important issues and represent one of the key points in the results comparison of various ASCs clinical trials [11].

Adipogenesis, fat survival and structure of adipose graft

The transplanted fat contains three types of adipose cells: ASCs, pre-adipocytes and adult adipocytes [4].

It seems that ASCs are responsible for both the adipogenesis and angiogenesis in the transplanted adipose tissue [9, 43], promoting the long-term survival of the fat graft [41]. The niche for ASCs [11, 12], important in determining cell fate and properties (phenotype, function, and life span) is found in the perivascular location of adipose tissue [12].

ASCs are considered a more reliable source than mature adipocytes, for inducing *de novo* lipogenesis [8, 10, 11, 39]. They can differentiate into mature adipocytes to form adipose tissue [41], and under normal metabolic conditions, they replace the dead cells and thus preserve the adipocytes number; this great turnover can renew about 10% of fat cells populations [10, 12]. It is known that the metabolic status influences the adipogenic properties of these cells; when there is an increased energy intake, ASCs multiply the number of adipocytes and expand the tissue [12]. ASCs also have the capacity to generate a new blood supply [41] by their paracrine secretion of different growth factors {*e.g.*, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and transforming growth factor beta (TGF-β) [43]}.

There are two stages of adipogenesis: transformation of multipotent ASCs into unipotent pre-adipocytes and

differentiation of pre-adipocytes into mature adipocytes [12].

Both the mechanism of ASCs transformation to the adipocyte lineage and the molecular phenotype of pre-adipocytes are under debate [12]. Many signaling molecules {*e.g.*, TGF-β, the fibroblast growth factor (FGF), the insulin-like growth factor-1 (IGF-1), the bone morphogenic protein (BMP), the activin and the interleukin-17 (IL-17) [12]} and several molecular markers, that enhance the adipogenic potential of the cells (*e.g.*, Lin-, CD_{24+}, CD_{29+}, CD_{34+}, stem cell antigen $(Sca\text{-}1)^+$, CD_{105-}, CD_{117-} or pericyte markers, $CD_{140\beta}$ protein, neural/glial antigen 2 (NG2) chondroitin sulfate proteoglycan, alpha-smooth muscle actin (α-SMA) or zinc-finger-protein 423}, are supposed to be involved [12].

On the contrary, the terminal differentiation of pre-adipocytes into mature adipocytes is well documented, implicating the activation of peroxisome proliferator-activated receptor-gamma (PPAR-γ), that promotes angiogenesis by upregulation of different adipogenic proteins [*e.g.*, adipocyte protein 2 (aP2) or leptin] [12].

The ASCs and the pre-adipocytes are the only cells that survive transplantation, since, due to the reduced metabolic activity and low oxygen consumption, they are more resistant to trauma and hypoxia [9, 44]. Numerous studies [9, 36, 43] have demonstrated that the final volume of adipose tissue graft is related to the survival of the ASCs in the SVF and to the remaining number of viable cells.

The adult adipocytes have a low viability because they are very vulnerable to trauma and ischemia, and die after transplantation [9, 44]. This explains the 20% to 70% reduction in volume of adipose tissue after transplantation [45].

In the structure of the newly implanted adipose graft, Eto *et al.* describes three zones: the central necrotic zone, the intermediate regenerating zone and the outer surviving zone [46].

In the central zone, in the first days after transplantation, the adult adipocytes undergo degeneration and necrosis due to ischemia; the macrophages and polymorphonuclear neutrophils are recruited and they phagocytose the cell debris.

The ASCs from the intermediate and the outer zones maintain their viability and, by proliferation and differentiation, replace the adipocytes in the necrotic zone [46] and initiate the revascularization [43]. Neoangiogenesis begins at the periphery of the graft and then, newly formed blood vessels vascularize the entire graft [4] (Figure 2).

Factors that influence the biological properties of ASCs

Several factors were found to influence the yield, the proliferation rhythm and the differentiation capacity of ASCs: anatomical locations, age, gender, and body mass index (BMI) [11–13, 38, 42, 47–52].

Two important zones of adipose tissue are described in humans: the subcutaneous fat (located in the abdomen, thighs, buttocks, and upper arms) and the visceral fat (in the perirenal, periintestinal, and omentum areas) [4, 12, 13]. These anatomical locations influence the long-term characteristics of fat grafts (the metabolic status: lipogenesis and lipolytic activity, fatty acid composition, the gene expression profile and the quality of cells [11, 12, 38]), both among individuals or within the same individual [11, 42], but they do not affect the number of processed viable ASCs [53]. Accordingly, ASCs from subcutaneous fat differentiate better into mature adipocytes than those from visceral fat [12, 38]; ASCs harvested from superficial abdominal subcutaneous regions (*e.g.*, above Scarpa's layer) are significantly more resistant to apoptosis (and therefore have a longer life span, but are less proliferative) than the same cells from deep subcutaneous areas [1, 10, 12, 38, 54].

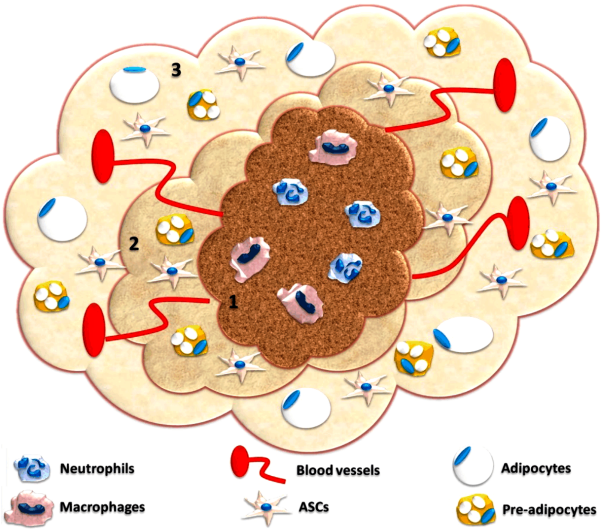

Figure 2 – ***The morphological changes in the adipose tissue graft. The degenerated adult adipose cells in the necrotic zone {1} are replaced by the proliferation and differentiation of the viable ASCs and pre-adipocytes in the regenerating zone {2} and the surviving zone {3}. The revascularization of the graft is initiated at the periphery and progresses towards the center. ASCs: Adipose-derived stem cells.***

Much more than fat location, the donor age may influence the biological properties of ASCs [10, 48, 50]. In their study, Schipper *et al.* [48] demonstrated (based on PPAR-γ as an indicator of adipogenic potential) an augmentation of adipose cells differentiation in all anatomical areas, in young people; in older patients, an increased differentiation was shown only at the level of arms and thighs. The same conclusion was reached in the study of Xu *et al.* [49].

Regarding the donor gender, numerous studies found that in the matter of osteogenesis, in men, the ASCs from superficial fat can differentiate more rapidly and efficiently into osteoblastes, when compared with the ASCs from the deep subcutaneous regions, or with the ASCs from all locations, in women [48, 50]; the same authors concluded that, in women, regardless of the adipose tissue location, there is no significant difference in bone formation [39, 48, 50]. This statement contravenes the study of Morena *et al.* [51], where it was demonstrated that in breast biopsies in women, the ASCs isolated from superficial subcutaneous regions have a better differentiation capability and a better response to osteogenic differentiation than the ASCs obtained from deep subcutaneous fat regions. van Harmelen *et al.* also found that, in women, the BMI is a very important factor, inversely correlated with ASCs differentiation [52].

SVF and ASCs

Adipose tissue is a complex structure, higly vascularized (each adipocyte possesses its own capillary network) and innervated (the autonomous nervous system controls the modulation of tissue features at molecular level) [10]. It contains mature adipocytes and SVF [5, 6, 8, 9, 13].

Adipocytes represent 90% of adipose tissue volume and nearly 65% of the entire cell population [9].

SVF is heterogeneous; it includes the ASCs (adherent subpopulation), but also fibroblasts, pre-adipocytes, monocytes, smooth muscle cells, endothelial cells, pericytes, B- and T-lymphocytes, hematopoietic-lineage cells [1, 2, 4–6, 8, 9, 11].

Zuk [13] claims that SVF could encompass at least three stem cell populations: the ASCs (CD_{34+}/CD_{31-}), the pericytes ($CD_{146+}/CD_{90+}/CD_{31-}/CD_{34-}$) and an endothelial precursor subpopulation (CD_{31+}/CD_{34+}) [5] (Table 4). All these populations share many of their surface profile markers [*e.g.*, aldehyde dehydrogenase (ALDH) and ABCG2 multidrug-resistance transport protein); still, the discrepancies regarding the presence of CD_{146} in each population, or the fact that SMA9 is specific only to the pericytes are all probably due to the isolation and expansion of distinct SVF cellular subsets [10, 13].

In order to obtain the ASCs, SVF must be purified [6, 41]. To remove the unwanted cells from SVF, two steps must be followed: firstly, the non-adherent cells are eliminated and secondly, the non-proliferative adherent cells are removed; by culturing or fluorescence-activated cell sorting, the non-adherent cells disappear; the rest of cells, still adherent to the culture dish, will lack the proliferative abilities, so they will be overgrown by the ASCs [6, 10, 41].

Table 4 – ***SVF expression of surface markers***

	SVF			
	Putative ASCs	***Endothelial progenitor cells***	***Vascular smooth muscle cells and pericytes***	***Hematopoietic cells***
Positive expression	CD_{90}	CD_{31}, CD_{34}, CD_{90}, CD_{146}	CD_{90}, CD_{146}	CD_{45}
Negative expression	CD_{31}, CD_{34}, CD_{45}, CD_{105}, CD_{146}	CD_{45}, CD_{105}	CD_{31}, CD_{34}, CD_{45}, CD_{105}	

SVF: Stromal vascular fraction; ASCs: Adipose-derived stem cells; CD: Cluster of differentiation.

Mostly in the United States (US) clinical studies, SVF is used in a non-purified state, since purification is considered a significant manipulation and requires the *Food and Drug Administration* (FDA) approval [6]). After harvesting, the lipoaspirate is processed in closed systems [6, 42] (Table 5). Before grafting, the lipoaspirate is frequently enriched with ASCs, supposed to exert a better retention and regeneration of the transplanted tissue [4, 6]. This clinical strategy is named cell-assisted lipotransfer (CAL) and it is based on two hypotheses: the ASCs improve tissue revascularization and fat survival, and they provide a matrix that helps the organization and differentiation of additional stem cells [39, 72–75]. There are still controversies about this method, mainly regarding the minimal cell dose/mL of graft necessary to induce a therapeutic effect [39].

Both ASC and SVF have the potential to be used in therapy [6, 41].

The use of autologous freshly isolated SVF cells is thought to be more advantageous (in matter of contamination, processing, clinical application, associated costs and foreign body reactions) than the use of culture-expanded ASCs [14], or even the allogeneic ASCs [41]. For example, SVF cells isolation is fast (within 60–90 minutes) and facile (performed in an operating room, using automated devices), and after an enzymatic digestion, about 200 000 cells/g of adipose tissue are obtained [39].

Table 5 – ***Enzymatic automated and semi-automated systems for isolation of ASCs***

Source of adipose tissue	Harvesting	Tissue preparation	Device	Cell isolation	Analyzed markers	Manufacturer	Mean No. of viable nucleated cells	Viability	Reference
Infrapatellar fat pad	Arthroscopic technique	Fat fractionization using syringe emulsification and concentration with an AdiPrep device	AdiPrep	Centrifugation, digestion with type I collagenase, flow cytometry analysis from each layer, SVF cells cultured for 10 days	CD_{45-}, CD_{44+}, CD_{73+}, CD_{90+}, CD_{105+}, CD_{166}, CD_{10}	Harvest Technologies	$4.86\pm2.64 \times 10^5$ cells/g (SVF cells)	69.03± 10.75%	[55]
Abdomen, thigh, or hip regions, male and female	Elective cosmetic surgery	Tissue digestion chamber, heating and agitation mechanism, and a three-stage filtration system	Automated system (patent pending)	Digestion with collagenase	CD_{31+}, CD_{34+}, CD_{73}, CD_{146} gene expression analyzed 18S, CD_{31}, CD_{34}, VEGF-A, IGF-1, HGF, v-WF, VE-cad	Patent pending	$1.17\pm0.5 \times 10^5$ cells/g (SVF cells)	96± 2.1%	[56]

Source of adipose tissue	Harvesting	Tissue preparation	Device	Cell isolation	Analyzed markers	Manufacturer	Mean No. of viable nucleated cells	Viability	Reference
Adult human subcutaneous adipose tissue	Simple aspiration with Toomey syringe/ waterjet-assisted aspiration (BodyJet; Human Med AG, Schwerin, Germany)	Closed system: processing canister, tissue washing, Celase reagent for enzymatic digestion, centrifuge chamber, the final cell product can then be aspirated from the chamber	Celution System	Celase® processing enzyme reagent	CD_{31}, CD_{34}, CD_{45}, CD_{90}, CD_{105}, CD_{146}	Cytori Therapeutics, San Diego, CA, USA	3.6±1.8 ×10^5 cells/g	84.7–93±2%	[57–59]
Lipoaspirates	Manual	Heated shaker and centrifuge inside of a sterile biohood with high-efficiency particulate air filtration and ultraviolet light which allows the entire processing to be conducted in sterile conditions	Multi Station	Solution composed primarily of type I and type II collagenases	CD_{31}, CD_{34}, CD_{45}	PNC International, Gyeonggi-do, Republic of Korea	1.07×10^5 cells/g	57± 21%	[59]
Lipoaspirates	Semi-automated	Custom disposable centrifuge syringes for the processing and handling of lipoaspirate	Medi-Kan Lipokit with MaxStem	Solution composed primarily of type I and type II collagenases	CD_{31}, CD_{34}, CD_{45}	Medi-Khan, West Hollywood, CA, USA	41.67% ASCs in the SVF 0.35×10^5 cells/g	72± 15%	[60–62]
Lipoaspirates	Semi-automated	Closed processing system	Cha-Station	Solution composed primarily of type I and type II collagenases	CD_{31}, CD_{34}, CD_{45}	CHA Biotech, Kangnamgu, Republic of Korea	0.05×10^5 cells/g	87± 12%	[60, 61]
Lipoaspirate	Automated	Lipoaspirate harvested directly into the system	GID SVF-1/ SVF-2	Proprietary enzyme mixture, GIDzyme-2 (GID Europe 2015)	CD_{105}, CD_{45}, CD_{90}, CD_{73}, CD_{14}, CD_{34}	GID Group, Inc.	719 000 nucleated cells/cm^3 of lipoaspirate or 7.19±2.11 ×10^5 nucleated cells/mL of dry adipose tissue	≥83%	[63, 64]
Usual liposuction	Automatic cell and tissue isolation device	Adipocyte isolation, SVF extraction, stem cell extraction, stem cell treatment, liposuction	Stem-X	Plant-based collagenase	n/a	Medikan Co., Ltd., Sasang-gu, Busan, Korea	n/a	n/a	[65]
Lipoaspirates	Fully automatic centrifuge to extract stem cells (SVF) from adipose tissue	Full automatic process for washing, purification, agitation of adipose tissue without contamination	Sceldis®	Type 1 collagenase	n/a	ED Co., Ltd. & Purebiotech Co., Ltd./ Medica Group	n/a	n/a	[66]

Source of adipose tissue	Harvesting	Tissue preparation	Device	Cell isolation	Analyzed markers	Manufacturer	Mean No. of viable nucleated cells	Viability	Reference
Lipoaspirate, elective cosmetic surgery	Automated, closed system for the isolation of SVF cells	Proprietary filtration system gravity (without centrifuge) enabled separation of fatty and aqueous fraction followed by filtration of the aqueous fraction to achieve SVF isolation and concentration	Stempeutron	Enzymatic digestion method	CD_{31}, CD_{34}, CD_{146}, CD_{73}, CD_{45}	Stempeutics Research Pvt. Ltd., Bangalore, India	0.89×10^5 to 2×10^5 cells/g of adipose tissue	94± 28%	[56, 67]
Subcutaneous abdominal fat liposuction	System performs only cell washing and concentration; tissue digestion must be performed manually	Liposuction samples in transfer bags (Terumo), added collagenase, incubator, after the bag is connected to a CS-490.1 kit (Biosafe SA), and installed on a Sepax device	Sepax and Sepax 2 systems	Ezymatic digestion (collagenase)	CD_{105}, CD_{90}, CD_{31}, CD_{34}, CD_{45}, CD_{73}	BioSafe America (Biosafe Group), Lake Geneva, Switzerland	260 000 nucleated cells/cm^3 of lipoaspirate processed $2.6\pm1.2\times10^5$ nucleated cells/mL of liposuction	>90%	[68]
Aspirated fat tissue (20–60 mL)	Automated special liposuction device with a filter (Lipivage, Genesis Biosystems, Lewisville, TX, USA	Automated SVF isolation processing chamber (tissue digestion, centrifugation and washing)	Tissue Genesis Icellator Cell Isolation system	Digestion – collagenase (Adipase™)	CD_{45}, CD_{31}, CD_{34}	Tissue Genesis, Honolulu, HI, USA	$7.02\pm1.89\times10^5$ cells/mL aspirated adipose tissue	78–80.7± 7.1%	[63, 69, 70]
Lipoaspirate	Semi-automated	Lipoaspirate to be harvested directly into the canister and centrifugation at 1000 g for 10 minutes	StromaCell by Microaire	Mechanical isolation	CD_{90}, CD_{44}, CD_{105}, CD_{73}	Microaire Aesthetics, Charlottesville, VA, USA	140 000 cells/cm^3 of lipoaspirate	87.3%	[71]

ASCs: Adipose-derived stem cells; SVF: Stromal vascular fraction; CD: Cluster of differentiation; VEGF-A: Vascular endothelial growth factor-A; IGF-1; Insulin-like growth factor-1; HGF: Hepatocyte growth factor; vWF: von Willebrand factor; VE-cad: Vascular endothelial cadherin; n/a: Not available. The Closed System Devices, produced by several companies, are semi-automated bioreactors, computer-controlled processing devices more advantageous for clinical use; they self-contained lipoaspirate, reducing the risk of contamination or human error during the culture period, standardize the cell isolation protocols and minimize processing times [6]; moreover, they collect, wash, digest, and separate cells without exposing them to the environment and the built in monitors control the cell viability, lactate production, pH/pO_2, and glucose levels [42].

Moreover, SVF enjoys the benefits of more progenitor cells [13, 41]. However, in their study, Kapur *et al.* [14] reported some disadvantages of SVF potency *vs.* expanded ASCs: insufficiency of ASCs/dose (the lipoaspirate giving a reduced number of cells for treatment [41]) or even a reduced/lack of efficacy [14, 41].

On the other hand, the controlled expansion of ASCs number [41], the controlled microenvironment for direct lineage differentiation, the possibility to monitor the adherence to a scaffold material, the selection of specific subpopulations [39] and the opportunity to establish an allogeneic cell line [41], are attractive reasons for the therapeutically use of ASCs in culture.

Little experimental data regarding the differences between SVF cells and ASCs are found in the literature [14], so their clear impact on regenerative medicine applications represents a matter of future investigations.

Regenerative features of ASCs

The restorative abilities of ASCs are largely appreciated for their therapeutic potential. These include: the capacity for proliferation and trilineage differentiation, the secretion of cytokines and growth factors, the immunosupression and immunomodulatory effects (Table 6) [6, 10, 12, 14].

Proliferative capacity

The proliferative capacity of the ASCs is higher than of the BMSCs and their survival can be enhanced by the over-expression of the catalytic subunit of the human telomerase gene [76]. The proliferation of the ASCs can also be increased by various cytokines and growth factors, such as the basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), IGF-1, tumor necrosis factor-alpha (TNF-α) and oncostatin M [8].

Table 6 – ***Regenerative properties of the ASCs***

Proliferation and differentiation	Secretome	Immunomodulation
▪ Mesenchymal lineages: osteogenic, chondrogenic, adipogenic, myogenic (skeletal, cardiac and smooth muscle); ▪ Ectodermal lineages: neurogenic; ▪ Endodermal lineages: hepatic, pancreatic, endothelial.	▪ Angiogenic factors: VEGF; ▪ Anti-apoptotic factors: IGF-1; ▪ Hematopoietic factors: CSFs and interleukins; ▪ HGF; ▪ TGF-*β*; ▪ Self-renewal supporting factors: FGF-2, LIF, fibronectin, vitronectin; ▪ Proliferating factors: EGF, PDGF, IGF-1, TNF-*α*, oncostatin M; ▪ Adipokines/hormones: leptin; ▪ ECM molecules: collagen type I, II, II, IV, fibronectin-1; ▪ ECM proteases: MMP-2; ▪ Other: thrombospondin-1, galectin 1, PAI-1, TIMP-1.	▪ Direct cell-to-cell interaction; ▪ Secretion of: LIF, PGE2, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, GCSF.

ASCs: Adipose-derived stem cells; VEGF: Vascular endothelial growth factor; IGF-1: Insulin-like growth factor-1; CSFs: Colony-stimulating factors; HGF: Hepatocyte growth factor; TGF-*β*: Transforming growth factor-beta; FGF-2: Fibroblast growth factor-2 (basic); LIF: Leukemia inhibitory factor; EGF: Epidermal growth factor; PDGF: Platelet-derived growth factor; TNF-*α*: Tumor necrosis factor-alpha; EMC: Extracellular matrix; MMP-2: Matrix metalloproteinase-2; PAI-1: Plasminogen activator inhibitor-1; TIMP-1: Tissue inhibitor of metalloproteinase-1; PGE2: Prostaglandin E2; IL: Interleukin; GCSF: Granulocyte-colony stimulating factor.

Differentiation

The *in vitro* and *in vivo* differentiation into multiple cell lineages is essential for cell-replacement therapy. Even though ASCs are of mesodermal origin, they can differentiate into non-mesenchymal lineages, of ectodermal and endodermal origin (Table 7) [77].

Several studies have shown this multipotential property of the ASCs and proposed *in vitro* protocols to be applied for obtaining the differentiation of the ASCs into specific phenotypes [6, 11, 78, 79].

The adipogenic differentiation was demonstrated by the intracellular formation of lipid vacuoles and was maintained after *in vivo* transplantation [80].

The differentiation into osteogenic and chondrogenic lineages was confirmed by the expression of specific markers and by the increased concentration of macromolecules characteristic for the extracellular matrix [81, 82].

The skeletal myogenic differentiation was proved by the expression of muscle-specific transcription factors and was also marked by the changes in cell morphology: cells became elongated and multinucleated, containing myofibrillar structures [83, 84].

The differentiation of the ASCs towards the neural lineage was confirmed by the expression of neuronal markers and the change in cell morphology [85].

Table 7 – ***ASCs differentiation***

	Cell lineage	Induction media	Lineage differentiation confirmation
Mesoderm	Adipogenic	IBMX, insulin, dexamethasone, MCGS, L-glutamine, indomethacin, ciglitazone, penicillin/streptomycin	Leptin, lipoprotein lipase, PPAR-*γ*2, Glut4
	Osteogenic	1,25-Dihydroxyvitamin D3, dexamethasone, ascorbic acid/ascorbate-2-phosphate, *β*-glycerophosphate, BMP-2, TGF-*β*1	Alkaline phosphatase, BMP-2, osteocalcin, osteogenic transcription factor Runx2, osteonectin, osteopontin, Osx
	Chondrogenic	Insulin, TGF-*β*1, TGF-*β*3, ascorbate-2-phosphate, BMP-4, bFGF, high-glucose DMEM, BMP-6, TGF-*β*3, dexamethasone, proline, pyruvate, ITS + premix (Becton Dickinson: insulin, transferrin, selenous acid, BSA, linoleic acid)	Type II collagen, aggregan, hyaluronan, GAGs
	Myogenic	Hydrocortisone, 5-azacytidine, bFGF	MyoD1, myogenin, myosin, dystrophin
	Smooth muscle	HUVECs culture media, 1% FBS, heparin, TGF-*β*1, BMP-4	*α*-SMA, basic calponin, SM-MHC, h-caldesmon
Ectoderm	Neurogenic	*β*-Mercaptoethanol, neurobasal medium containing B27 with FGF and EGF, IBMX and dibutyryl cAMP or forskolin and butylated hydroxyanisole, valproic acid, forskolin, hydrocortisone, insulin, DMEM with human serum, glutamine, penicillin, streptomycin, amphotericin, indomethacin	Neurogenic proteins: NeuN, GFAP, MAP-2, NSE, nestin, *tau*, *β*-tubulin, Nkx2.2, Pax6, Olig2
Endoderm	Endothelial	Medium 199, VEGF, bFGF, 3% FBS, EGM-2, endothelin-1	CD_{31}, CD_{34}, VE-cad, CD_{144}, vWF
	Hepatic	EGF, activin A, bFGF, HGF, nicotinamide, knockout serum replacement, DMEM serum-free, DMEM-LG, FBS, dexamethasone, ascorbic acid 2-phosphate, insulin, transferrin, sodium selenite, DMSO, OSM	
	Pancreatic islet-like cells	DMEM (60%), MCDB 201 (40%): EGF, dexamethasone, ascorbic acid, FBS, knockout DMEM (FBS, glutamine, non-essential amino acids), ITS, fibronectin-supplemented DMEM/F-12, DMEM/F-12 with N-2 supplement, B-27 supplement, bFGF, glucose-free nicotinamide, exendin-4	

ASCs: Adipose-derived stem cells; IBMX: 3-Isobutyl-1-methylxanthine; MCGS: Mesenchymal cell growth supplement; BMP: Bone morphogenetic protein (-2, -4, -6); TGF: Transforming growth factor (-*β*1, -*β*3); bFGF: Basic fibroblast growth factor; DMEM: Dulbecco's modified Eagle's medium; ITS: Insulin-transferrin-selenium; BSA: Bovine serum albumin; HUVECs: Human umbilical vein endothelial cells; FBS: Fetal bovine serum; EGM-2: Endothelial cell growth medium-2; EGF: Epidermal growth factor; cAMP: Cylic adenosine monophosphate; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte growth factor; LG: Low glucose; DMSO: Dimethyl sulfoxide; OSM: Oncostatin M; PPAR-*γ*2: Peroxisome proliferator-activated receptor-*γ*2; Glut4: Glucose transporter type 4; Runx2: Runt-related transcription factor 2; Osx: Osterix; GAGs: Glycosaminoglycans; MyoD1: Myogenic differentiation 1; *α*-SMA: Alpha-smooth muscle actin; SM-MHC: Smooth muscle-myosin heavy chain; NeuN: Neuronal nuclei; GFAP: Glial fibrillary acidic protein; MAP-2: Microtubule-associated protein-2; NSE: Neuron-specific enolase; Pax6: Paired box 6; Olig2: Oligodendrocyte transcription factor 2; CD: Cluster of differentiation; VE-cad: Vascular endothelial cadherin; vWF: von Willebrand factor.

ASCs secretome

The secretome of the ASCs includes: cytokines, growth factors, extracellular matrix proteases, hormones and lipid mediators. These soluble factors ensure the tissue regeneration *via* paracrine mechanisms. The angiogenic factors, such as VEGF, increase capillary density and promote the vascular supply in the ischemic tissues. Moreover, ASCs can act as pericytes and thus they can control the microvascular density and stability [14]. The anti-apoptotic factors (such as IGF-1), the hematopoietic factors [such as colony stimulating factor (CSF) and IL], the HGF and TGF-β facilitate the wound healing and the tissue repair at the site of the injury [12]. Extracellular matrix (ECM) molecules and proteolytic enzymes are involved in the histogenesis of the adipose and connective tissue, wound repair and cell migration. Tissue inhibitor of metalloproteinase-1 (TIMP-1) controls the activity of matrix metalloproteinases (MMPs) and stimulates cell proliferation. Thrombospondin-1 and galectin-1 mediate the interactions between the cells and the ECM, and plasminogen activator inhibits fibrinolysis [6].

Moreover, the intrinsic secretion of self-renewal supporting factors including FGF-2, leukemia inhibitory factor (LIF), fibronectin and vitronectin, which act as feeders, enables the reprogramming of the ASCs into induced pluripotent stem cells (iPSCs), even in the absence of helping feeder cells [86].

Engineered ASCs can be transformed in biopumps that multiply the innate capability to secrete factors in their host environment. Accordingly, increased levels of BMP, FGF-2, VEGF and brain-derived neurotrophic factor (BDNF) can be obtained while stimulating ASCs osteogenic and angiogenic properties [14].

Immunosupression

The immunosupression of activated immune cells exerts significant immunomodulatory effects accomplished by direct cell-to-cell interaction or by the secretion of various biomolecules: LIF, prostaglandin E2 (PGE2), increased levels of IL-4, IL-6, IL-10, granulocyte-colony stimulating factor (GCSF), TNF-α or leptin. GCSF, IL-6, IL-7 and IL-11 have pro-inflammatory effects, and IL-8 is chemoattractant for neutrophils and basophils. TNF-α recruits and activates neutrophils and monocytes. Leptin regulates the activity of various cells, including immune cells, adipocytes, muscle cells, blood cells and pancreatic β cells [6]. Additionally, ASCs promote the proliferation of regulatory and T-helper lymphocytes [14].

The modulation of the ASCs target environment due to the paracrine secretion of all these biomolecules is known as the "bystander" effect [14] and recently has been used for preventing or treating autoimmune and inflammatory diseases [6, 12, 14]. Moreover, the absence of the MHC class II enables the allogeneic transplantation of ASCs without significant immune reaction in the recipients [87].

Administration of ASCs

There are different routes to deliver the ASCs to the injury sites. It is less known what percentage of ASCs actually migrates to the targeted damaged tissues [88]; therefore, to insure the optimal clinical efficiency, all routes must be attentively considered before their application [41] (Table 8).

Clinical strategies

In order to enhance the clinical efficacy of ASCs therapy, many aspects have to be considered. Every step presented insofar can be improved and further investigated, although the research focuses on cells survival, differentiation and migration site. Engineering, allogeneic, and cell-free therapies or the possibility to use iPSCs are in constant development as future therapeutic targets.

Homing of ASCs

Regionally or systemically injected ASCs are capable to migrate towards injured or diseased tissues, phenomenon known as "homing"; this feature is due to the chemoattractant effect of cytokines and growth factors [*e.g.*, EGF and monocyte chemoattractant protein-1 (MCP-1)] produced by the cells of the targeted tissues that interact with the ASCs surface receptors [6]. Unfortunately, the "homing" of ASCs in systemic delivery can only be partially obtained, and the risk of cells accumulation in the lungs is high (Table 8). Because ASCs belong to the BMSCs class, clinical strategies are generally translated from the BMSC research (Table 9) [41].

Engineering (pre-conditioning)

Most autologous transplants have a poor outcome; they are gradually but largely resorbed, being replaced by conjunctive tissue with fat cysts [10, 45]. The decreased adipocytes tolerance to ischemia and the low rate of revascularization are the main implicated factors [9, 10, 44].

Table 8 – ***Different routes for ASCs delivery***

Delivery		Mechanisms	Results	Risks
Systemic delivery	Intravenous	Depends on the native "homing" of ASCs to the injury site (cell surface receptors allow ASCs to migrate by chemotactic mechanisms promoted by local inflammation) [88–90]	Enhanced graft efficiency in autoimmune diseases	Leads to accumulation in the lungs (mainly), liver, brain, heart [88]
	Intraarterial			Augmented number of ASCs could lead to pulmonary emboli or infarctions or might alter the blood flow in different other tissues, if the quantity of ASCs is increased for the purpose of better outcome [91]
	Intraperitoneal			
	Intracardial			
Local administrations	*In situ* injections	Enhanced graft efficiency in wound healing, bone regeneration		Invasive procedure (*e.g.*, injection into the myocardium) Cells are directly injected into a potentially incompatible microenvironment

ASCs: Adipose-derived stem cells.

Table 9 – *Strategies that enhance the "homing" of ASCs*

Strategy	Mechanism
The use of *freshly* isolated cells	The expression of cell surface chemokine receptor CXCR4 in culture is altered
To induce and enhance *in culture* the expression of cell surface chemokine receptor CXCR4	The use of a cytokine cocktail that promote stem cells migratory capabilities Hypoxic conditions Viral transduction (modification of the gene expression of cell surface receptors) Pre-conditioning

ASCs: Adipose-derived stem cells; CXCR4: C-X-C motif chemokine receptor 4.

Due to their proliferative abilities, ASCs intensively participate in tissue morphogenesis and remodeling [4, 7, 10], therefore being targeted for engineering techniques.

ASCs manipulation before administration boosts all their capabilities, including their paracrine effects, with great impact on clinical efficacy [41].

The ASCs engineering, favors two directions: the gene/cell therapy and tissue engineering [11].

In gene/cell therapy, autologous ASCs, expanded *in vitro* and transduced with a therapeutic gene viral vector carrier, are implanted in the recipient to regenerate damaged tissue (Table 10) [11].

In tissue engineering, autologous ASCs, expanded *in vitro* and combined with viable biomolecules and biomaterials are implanted in the recipient to generate an *ex vivo* tissue [11]. Specific growth factors and various additives are used in order to direct the differentiation into the desired lineages before implantation (Table 7) [39].

SVF is seeded on various biocompatible scaffolds and complex matrices that enable cell attachment and direct differentiation *in vivo* [6]. The combination of the biological component, represented by the stem cells, and the artificial scaffolds, promotes the long-lasting repair [38]. There are many strategies for obtaining an adipose tissue engineering construction (Table 11) [6, 10].

The scaffolds used for tissue engineering must fulfill certain criteria: they are biocompatible (creating a micro-environment that allows cell attachment, matrix cell migration, proliferation and differentiation of the ASCs), biodegradable (at a slow pace, for preserving the structural integrity of the matrix), adaptable to the three-dimensional architecture of the defect (resistant to complying with the contraction forces of the lesion and structural remodeling and spacious enough for the developing of the new tissue and its new vascular network) and easily reproducible [10, 11]. The biomaterials used to create the scaffolds are natural and synthetic polymers. Their advantages are presented below (Table 12) [10].

Table 10 – *Methods of ASCs pre-conditioning*

Method	Type of virus	ASCs capability	Therapeutical target	Associated risk and discussions
Molecular stimulation (combination of gene therapy and stem cell therapy) ▪ Gene delivery vector (transduction of the therapeutic gene with replication-defective viral vectors (retro-, lenti-, adenoviruses) into the DNA of the recipient cells) [11, 41]	Retrovirus	Enhanced expression of growth factors [41]	Wounds [41]	Tumorigenesis (retro/lentiviruses, that integrate into host genome) [41] (daunting regulations for human translational clinical trials [42]
	Lentiviral vector carrying *PDX1* gene [41]		Diabetes (mice) [41]	
	Lentiviruses [11]	Differentiation into adipogenic and osteogenic lineages [11]	*In vitro* studies [11]	
	Lentiviral vector carrying TRAIL–cDNA [11]	Apoptosis of A375 melanoma cells [11]	Malignant melanoma (mice) [11]	
	Lentiviral vector carrying *cIFN* gene [11]	Inhibition of the growth of canine melanoma LMeC cells [11]	*In vitro* study [11]	
	Lentiviral vector expressing the *α*1-AT [11]	Reduction of bone loss [11]	Bone loss (ovariectomized mouse model) [11]	
	Lentiviral vector encoding human HGF [11]	Differentiation into endothelial cells (increased blood flow and decreased fibrosis) [11]	Acute myocardial infarction (rat model) [11]	
	pCDH813A-1 lentiviral vector carrying *RIL-23R* gene [11]		Basic research (*in vitro* study) [11]	
	Lentiviral vector carrying the cytotoxic T-lymphocyte antibody [42]		Autoimmune thyroiditis (mice) [42]	
	Adenovirus	BMP-2 expression [41]	Bone formation (immunodeficient mice) [41]	Adenoviruses (they do not integrate into host genome) [41, 42] Safer alternative
	Adenovirus vector carrying *α*1-AT [42]		Liver (mice) – inborn metabolic errors [42]	
Genetic stimulation ▪ Use of genetic knockdown by short hairpin RNA (inhibit PDE-5) after intramyocardial injection	Enhanced paracrine activity		Myocardial infarction	

ASCs: Adipose-derived stem cells; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; PDE-5: Phosphodiesterase-5; PDX1: Pancreatic and duodenal homeobox 1; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; cDNA: Complementary DNA; cIFN: Canine interferon; *α*1-AT: Alpha 1-antitrypsin; HGF: Hepatocyte growth factor; RIL-23R: Recombinant interleukin-23 decoy receptor; BMP-2: Bone morphogenetic protein-2.

Table 11 – ***Biomaterials and adipose tissue engineering techniques***

Applications for tissue formation	Biomaterials	Biological effects	References
Adipogenesis	Matrigel (a collagen-based gel derived from the basement membrane of a murine tumor) with bFGF	Formation of a tissue mass consisting of high triacylglycerol content	[92, 93]
	Absorbable polymeric scaffolds consisting of PLGA and HA-based scaffolds used as temporary "space fillers"	Cellular proliferation and scaffold resorption resulting in mature adipose tissue	[94, 95]
	ECM hydrogel, composed of soluble ECM obtained by the centrifugation of human adipose tissue and methylcellulose	Formation of a new and functional adipose tissue	[96]
Osteogenic	Natural, synthetic, and nanocomposite biomaterials Biodegradable PLA nanoparticles PCL	Endochondral ossification and formation of bone with bone marrow	[97–99]
Tenogenic	Decellularized ECM	Unsatisfactory clinical results	[100]
Chondrogenic	Natural polymer: HA	Regeneration of the articular cartilage	[101]
Neural	MWCNTs inserted in the PDMS/MWCNT and a mixture of glial growth factors	Neuronal proliferation, formation of a long neurite and synergistic effects in the regeneration of peripheral nerves	[102]
	Hydrogel gelatin tubes	Axon regeneration, myelin formation and restoration of the denervation	[103]
Skeletal muscle	Collagen hydrogel	Myogenic proliferation	[104]
Smooth muscle	PPy and electrical stimulation	Vascular smooth muscle regeneration	[105]
Cardiac muscle	Scaffolds consisting of polyacrylamide hydrogel coated in turn of collagen were synthesized	Blockage of the differentiation of cardiac myofibroblasts and prevention of cardiac fibrosis	[106]
Endothelial	Nanofibrous structure of PCL/gelatin ELR-based hydrogel	Regeneration of blood vessels Formation of functional microvasal structures	[107]
Pancreatic tissue	Autologous growth factors and nanofibrous scaffolds	*β*-Cell replacement for therapy of type 1 diabetes mellitus	[108]

bFGF: Basic fibroblast growth factor; PLGA: Poly(lactic-*co*-glycolic acid); HA: Hyaluronic acid; ECM: Extracellular matrix; PLA: Poly(lactic acid); PCL: Poly(*ε*-caprolactone); MWCNT: Multi-walled carbon nanotube; PDMS: Poly(dimethylsiloxane); PPy: Poly(pyrrole); ELR: Elastin-like recombinamer.

Table 12 – ***Advantages of natural and synthetic polymers***

Polymers	Advantages
Natural	Biocompatible Hydrophilic Biological/mechanic properties are similar with living support
Synthetic	Mechanic/chemical/degradability properties consistent with targeted tissue characteristics but adjustable

Engineered ASCs in coadministration with drugs or biomolecules have many advantages in cancer, where they may decrease the toxicity of the drugs in use; within ASCs as carriers, a lower dose of medicine can be applied [41]. The effectiveness of this treatment was demonstrated in preclinical studies [11]. For example, Argentati *et al.* [11] described two animal models where engineered ASCs were combined with low doses of chemotherapeutic agents (Cisplatin and 5-Fluorocytosine) to successfully treat leukemia, colon cancer and glioblastoma.

The "homing" ability of ASCs combined with their possible pre-conditioning led to the emergence of a new therapeutic branch, the stem cell-mediated suicide gene therapy [41]. It is a safe procedure, since after finishing their task, ASCs are eliminated [41]. Genetic engineered ASCs secrete an enzyme that transforms the coadministrated biomolecule into a tumor cytotoxic factor; the ASCs "bystander" effect destroys the neighboring area [41].

Recently, *in vitro* and *in vivo* preclinical studies reported effective results by applying this procedure for the treatment of cervical, breast, colon and pancreatic cancer, brainstem glioma or glioblastoma [41].

Allogeneic ASCs transplantation

Another modern regenerative medicine strategy targets allogeneic ASCs transplantation.

ASCs are immune-privileged cells, that generate no or minimal immune responses, when transplanted to normal immune subjects. They owe their immunomodulatory properties to the lack of MHC class II expression and to the costimulatory molecules from their surface [6, 12, 41]. In order to obtain this feature, ASCs must be purified from heterogeneous SVF that exerts a T-lymphocyte response [6].

Allogeneic cell transplantation has multiple benefits: cells from a single donor can treat multiple patients and can improve fat harvesting, donor variance, loss of cells function, development of unwanted cell types or tumorigenesis [41]. Allogeneic cells can be immediately available for patients and may assure the standardization of a potentially therapeutic product; healthy donors can be preselected, based on their molecular profiles [41]. Transplanted allogeneic cells represent a major advantage for clinical trials: they can insure the comparisons between the efficacies of the stem cells used in the cohort or between the therapeutic benefits of the allogeneic *vs.* autologous graft in each trial [41].

The location where this procedure can be applied represents the main disadvantage. Centers with rigorous monitoring are needed in order to insure the FDA standards regarding cells significant manipulation (for obtaining the high number of ASCs requested in each clinical trial, cells must be expanded in culture) [6].

Several pharmaceutical companies (*e.g.*, Tigenix Belgium) are involved in producing allogeneic ASCs

products for the treatment of different disorders (*e.g.*, osteoarthritis, perianal fistula, graft *vs.* host disease, diabetes with limb ischemia, mild and severe heart failure, and ischemic stroke) [12, 41]. Six clinical trials using allogeneic transplantation in different phases of development are enlisted on the *NIH* site in 2018 [11].

Cell-free approach for therapy (conditioned medium)

Conditioned medium represents a cell-free therapy, based on the enhanced therapeutic power of ASCs secretome [6]. It is an alternative to ASCs direct transplantation and can be obtained by standardized and reproducible protocols [6].

The procedure uses the extracellular release of genetic material and biomolecule-rich vesicles by the ASCs endosomal compartment. These vesicles preserve the initial properties of stemness [41].

Several preclinical animal studies have outlined its high efficacy, successfully tested in a broad spectrum of disorders: inflammatory diseases, acute injuries (liver, lung, kidney, and skin), or cardiovascular illnesses [41]; yet, future investigations are still needed, before considering the clinical translation.

However, the following benefits are observed when compared to the stem cell therapy: a lower risk for an immune response in patients, reduced undesired paracrine effects, safe use of vesicles (without concerns of tumorigenicity), long-term preservation of vesicles without the loss of their function, and fewer regulations to be applied in clinical trials.

The disadvantages are represented by insufficient safety data about all paracrine secreted factors and their long-term effects [13, 41].

iPSCs

A stem cells population with embryonic abilities, easily obtained and without ethical burden may be of great utility for clinical trials. Accordingly, another research avenue is taken into consideration: the iPSCs, which possess the abilities of embryonic stem cells (self-renewal and multilineage differentiation capacity) [11, 14].

Human iPSCs are obtained from different somatic cells with various genes profiles. *In vitro* iPCS colonies form embryoid bodies which express different germ layers markers: glial fibrillary acidic protein (GFAP) – ectoderm; α-fetoprotein, SRY (sex determining region Y)-box 17 (SOX17), pancreatic and duodenal homeobox 1 (PDX1) – endoderm; vimentin, desmin, SMA – mesoderm [13, 14]. Classic stemness gene expression profiles [octamer-binding transcription factor 4 (*OCT4*), *SOX2*, Krüppel-like factor 4 (*KLF4*), *cMYC*] and additional pluripotent cell surface markers [*e.g.*, stage-specific embryonic antigens (SSEAs) and TRA] are now recognized for the iPSC lines. Still, a more unifying genetic phenotype is needed in order to obtain clinical efficiency and reliable therapy products [11].

Fresh and cryopreserved sources of human and animal (pig, dog, and mouse) commercial ASCs lines are used for the transformation into iPCS [13, 14].

In matter of therapy, the advantage of iPSCs procedure consists in deciphering the molecular basis of given diseases *in vitro*, and designing the personalized effective therapeutic drugs [12]. Studies already confirmed this potential in myocardial infarction and limb ischemia therapeutic models [13]. Still, concerns are rising regarding the possible occurrence of teratomas, or genomic instability (apparently resulting from the transfection with *cMYC* oncogene). Another disadvantage is the limited number of iPSCs colonies able to expand *in vitro* [13].

Further research is still needed in order to surpass the existing limits.

Dedifferentiation of mature adipocytes (DFAT cells)

Dedifferentiation of mature adipocytes back to progenitor cells produces a new source of ASCs; therefore, it is considered a new future direction in therapy [13, 14].

It is not yet known if these progenitor cells are true ASCs or a similar primitive population [13, 14].

The first hypothesis is supported by dedifferentiated fat (DFAT) cells properties, confirmed by *in vitro* studies: same ASCs CD profile, same potential of multilineage differentiation, immunosuppressive abilities and similar expression of stemness genes (*OCT4*, *SOX2*, *cMYC* and *NANOG*, *SSEA1*). Still, all these features are found at another purity level than in common ASCs [13, 14].

However, this new source of ASCs, beside the one found in SVF fraction, helps to increase the pool of multipotent stem cells available for clinical therapy [13].

ASCs for somatic cell nuclear transfer (SCNT)

It is a small area of research that encompasses reprogramming ASCs *via* SCNT.

It is applied for *in vitro* fertilization or cloning.

There are few literature studies, but they generally outline as nuclear donors for SCNT embryos, either the resistant porcine ASCs lines or the DFAT primitive population cells [13].

Immortalized ASCs lines

Produced for the first time in South Korea, immortalized ASCs lines are obtained by transduction with the catalytic subunit of the human telomerase gene. The benefits of the strategy are: enhanced longevity in culture and *in vivo* with normal karyotypes, and increased differentiation potential irrespective to the engraftment zone [13].

Cryopreservation

Still a niche area of research, cryopreservation seems to be the safest procedure until now, regarding ASCs archiving and biobanking [109–112]. It may insure the permanent availability of these cells and their immediate delivery to the providers [5].

In culture, cryopreserved ASCs maintain unaltered their stemness properties (viability, gene expression, differentiation capacity and immunophenotype markers) despite multiple rounds of freezing-thawing; by archiving them, donor variables are avoided [11, 109–112].

There are many cryopreservation agents; the classic dimethylsulfoxide (DMSO) is the only one used in combination with serum protein components, a reason why it may induce potential adverse reactions in the

recipient; the new agents, such as polyvinyl, trihelose and hydroxyl ethyl starch, are used under serum-free conditions and are safer [5].

Cell products are kept in storage containers, either submerged in liquid nitrogen at -70°C/-80°C or better, in vapor-phase liquid nitrogen (to remove cross-contamination) [5]. Cryopreservation of adult stem cells protocols still require improvements for the elimination of contaminants, animal serum or cryoprotectant additives used in the freezing of these cells [113–115].

More data over these alternative options should be collected and validated in order to be used as future production standards [5].

Harvesting, isolation, and processing methods of ASCs

Although recent studies unanimously agree on the harvesting procedures of adipose tissue, there is not yet a standardized protocol for ASCs isolation for clinical applications [9]; therefore, there are many inconsistencies in literature data (Table 13).

Table 13 – ***Beginning history of ASCs isolation and characterization***

Author, year	Animal/human	Type of cell isolated	Cell properties	Reference
Rodbell, 1966	Rodents	Progenitor cells from adipose tissue	Anabolic processes stimulated by insulin and phospolipase C	[116]
Van & Roncari, 1977	Adult rats' adipose tissue	Cells isolated from the stromal fraction	Cells with a proliferative rate and morphological characteristics similar to adipocytes	[117]
Deslex *et al.*, 1987	Human inguinal fat tissue	Stromal vascular cells	Cells undergo adipose conversion in culture with insulin, transferrin and triiodothyronine	[118]
Hauner *et al.*, 1989	Adult human subcutaneous adipose tissue	Stromal vascular cells	Terminal adipose differentiation % of differentiated cells depending by donor age	[119]
Williams *et al.*, 1994	Liposuction-derived human fat	Adipocytes and endothelial cells	Histochemistry studies revealed the expression of von Willebrand factor	[120]
Moore *et al.*, 1995	Syringe suction lipectomy	Adipocytes isolated through collagenase digestion	Fully viable and functional adipocytes	[121]
Katz *et al.*, 1999	Subcutaneous adipose tissue	Procurement of autologous cells	Emerging tissue-engineering strategies for fat	[122]
Pittenger *et al.*, 1999	Bone marrow-derived adult stem cells	Human mesenchymal stem cells	Clonal analysis	[123]
Sen *et al.*, 2001	Human adipose tissue stromal cells	Stromal cells (pre-adipocytes)	Expression of an adipocyte specific gene product aP2 Cells isolated from multiple donors present different degrees of differentiation	[124]
Zuk *et al.*, 2001	Human adipose tissue by suction-assisted lipectomy	Fibroblast-like cells or processed lipoaspirate cells	Mesenchymal origin cell + low levels of pericytes, endothelial cells, and smooth muscle cells Lineage-specific differentiation: adipogenic, chondrogenic, myogenic, osteogenic, and neurogenic	[3, 125]
Halvorsen *et al.*, 2001	Human liposuction	Human adipose tissue-derived stromal cells	Osteoblast differentiation	[126]
Gronthos *et al.*, 2001	Human liposuction	Undifferentiated and differentiated adipose tissue-derived stromal cells	Cells cultured under adipogenic conditions	[127]
Mizuno *et al.*, 2002	Human lipoaspirates	Processed lipoaspirate cells	Myogenic differentiation	[83]
Safford *et al.*, 2002	Murine, human stromal cells	Adipose-derived adult stem cells	Neuronal differentiation	[85]
Gimble & Guilak, 2003	Rodent lipoaspirate	Adipose-derived adult stem cells, new protocol	Confirming cell's multipotency activities	[128]
Miranville *et al.*, 2004	Human adipose tissue	SVF	Characteristics of endothelial progenitor cells improve vasculogenesis	[129]

ASCs: Adipose-derived stem cells; SVF: Stromal vascular fraction; aP2: Adipocyte protein 2.

Adipose tissue harvesting

Adipose tissue harvesting can be performed through both lipectomy and liposuction; solid fat tissue and respectively liquid fat tissue are obtained. Liposuction can be performed through either vacuum-aspiration or syringe-aspiration. Pu *et al.* [35] found that vacuum-aspiration (conventional liposuction), compared to lipectomy fresh samples or syringe-aspiration, significantly impaired adipose cells function, causing structural damage in up to 90% of adipose cells [130, 131]. However, vacuum-aspiration (or low negative pressure aspiration) is faster than syringe-aspiration and can be used when there is a need for a large volume of adipose tissue (*e.g.*, in breast surgery).

The size of the cannulas used in liposuction can also influence the viability of cells; large-bore cannulas and lipectomy reduce the cellular damage and preserve the tissue architecture [132]. Apparently, there is an inverse correlation between the diameter of the harvesting instrument and the cellular damage; higher graft viability is reported with the use of 6 mm cannulas (compared to 2 mm or 4 mm cannulas) [133, 134]. In 2006, Coleman *et al.* introduced a manual procedure involving the use of a 3 mm, blunt-edged, two-hole cannula; in order to obtain adipose cells, this is connected to a 10 mL syringe for harvesting, followed by centrifugation [36]. For the treatment of delicate areas (such as eyelids or lips), very small cannulas (0.7–1 mm) can be used in the so-called nano or micrografting procedures [135, 136]. The nano-grafts contain good amounts of ASCs, despite possessing

few or even no adipose cells (compared to micrografts or macrografts); this makes them suitable in clinical practice for skin rejuvenation [137, 138].

Adipose tissue harvesting can be performed from different areas of the human body: most frequently, the abdomen, the trochanteric region, the inside of knees and thighs [10, 139, 140]. There are two ways to obtain the grafts: the "wet" and the "dry" methods, showing no differences concerning cell viability and characteristics [141]. The "wet" procedure involves the injection of the harvesting site with Klein's solution (containing local anesthetic, epinephrine and 0.9% sodium chloride) [142]; the "dry" method is based on general anesthesia or regional blocks [28, 130, 142].

After harvesting, the adipose tissue has to be processed in order to eliminate blood, collagen fibers and cellular debris; all these elements can cause inflammation at the injection site, degradation of the graft (due to the presence of blood), or decreased graft volume after several hours (due to the resorption of cellular debris) [7, 18, 143].

Isolation of ASCs after liposuction

In 2001, Zuk *et al.* were reporting for the first time the isolation of ASCs after liposuction using enzymatic methods in use even today [125]; in order to remove blood cells, saline and anesthetic, the lipoaspirate is washed with sterile phosphate-buffered saline (PBS); then, 0.075% collagenase type I is applied for digestion; inactivation of enzymatic activity is performed with an equal volume of 10% fetal bovine serum (FBS), Dulbecco's modified Eagle's medium; finally, erythrocytes lysis is performed and ASCs are separated through centrifugation. This technique, although very effective and commonly used, is also complex, time-consuming (2 hour-long) and expensive for regular clinical use [2, 7, 144].

More recently, Raposio *et al.* compared two methods of ASCs isolation: a mixed mechanical and enzymatic (ME) technique with only pure mechanical (MC) technique [9, 145].

The ME procedure involves centrifugation and collagenase digestion; after liposuction performed with a 4 mm cannula, the tissue is centrifuged at 1600 rpm, for 6 minutes, to obtain approximately 50 mL of high quality adipose tissue; than 50 mL of diluted collagenase is added (1 g of collagenase is first suspended in 10 mL of PBS and then 1 mL of this solution is further diluted with 49 mL of PBS); the resulting solution is incubated at 37°C, for 30 minutes, in a shaker-incubator and then, centrifuged again at 400 rpm, for 4 minutes, to obtain approximately 10 mL SVF; two washing cycles (with 45 mL saline solution) and a new centrifugation (at 400 rpm for 4 minutes) followed, in order to obtain the ASCs pellet, resuspened in 5 mL saline solution. This entire procedure is performed in a closed-circuit system that guarantees sterility and safety.

The MC procedure involves liposuction with a 4 mm cannula, that first introduces Klein solution at the site of aspiration; 80 mL of sample in 10 mL-plastic test tubes are subsequently obtained; then, the tubes are placed on a vibration shaker at 600 vibrations/min for 6 minutes, and immediately centrifuged at 1600 rpm for 6 minutes; the pellet at the bottom of each test tube is afterwards collected with an automated pipetting system and transferred into a 10-mL Luer-Lok™ syringe; this procedure is performed entirely under a laminar air-flow bench and lasts approximately 15 minutes.

By comparing the two methods, Raposio *et al.* [145] reported a 99% vitality of cells in both techniques; however, a higher percentage of ASCs in the lipoaspirate using the ME technique was observed (25.9% *vs.* 5%). Still there are some disadvantages: the ME procedure requires 80 minutes *vs.* 15 minutes for the MC procedure and also, the use of collagenase for digestion increases the risks of clinical side effects (*e.g.*, cutaneous ulcers, nerve/tendon/ligament damage, or allergic reactions); the residual collagenase activity in human samples was not insofar analyzed, although Chang *et al.* [146] reported a nonexistent level after several washing steps in mice. No studies have also focused on the safety of FBS in clinical practice.

Another new ASCs isolation technique from lipoaspirate, based on antibody-coated immune-magnetic spheres, was recently described by Rada *et al.* [147].

Processing the lipoaspirates

The most common methods used for processing the lipoaspirates are sedimentation, filtration, washing and centrifugation [7].

Animal studies revealed no differences in the weight or architecture of grafts after centrifugation, filtration or sedimentation, but human studies show that centrifugation has better outcomes than sedimentation; still, in humans, centrifugation, filtration and washing have the same fat retention [7].

Sedimentation, although less traumatic and with a high yield of vital and intact adipocytes, has a lower concentration of stem cells and a higher contamination percentage with blood cells, lipids and aqueous fraction with proinflammatory effects [148, 149].

Filtration might be more effective when higher volume is needed; also, it allows elimination of contaminants and a higher percentage of ASCs and adipose cells [149]. According to some authors [4], the new technology that uses a closed-membrane filtration system might prove more beneficial than centrifugation (although when compared to older filtration techniques, no differences were found) [150].

Washing of lipoaspirate was proven to yield a higher percentage of stem cells and adipocytes, and to eliminate effectively the contaminants [148].

Centrifugation still remains the most widespread used procedure. At more than 50 g, centrifugation can cause damage to the tissue architecture (*i.e.*, increased necrosis and apoptosis, decreased cell differentiation capacity and tubule formation [151] and also increased fluid fraction, reduced injectable tissue volume and increased oil fraction linked to adipose cells damage) [152, 153]. Recently, Ferraro *et al.* reported that centrifugation at 250 g for 5 minutes is preserving cell viability and yields an increased concentration of stem cells [4, 151]. The widely used 2006 Coleman technique involves centrifugation of aspiration syringe at 3000 rpm for 3 minutes; this produces three fractions: the upper layer (containing lipids), simply eliminated by pouring it on an absorbent material; the medium layer (containing adipose tissue) and the lower layer (containing blood, tissue fluid and anesthetic), removed through the base of the syringe [36].

After processing, the adipose graft is injected at the injury site through skin incision and use of the cannula [7]. Depending on the recipient site, many authors suggest the use of different caliber cannulas; some authors observed a higher vitality of adipose tissue when using minimum 2.5 mm diameter cannulas [132], while others observed no influence [134]. It seems that, a smaller diameter of the cannula lowers the risk for bleeding, hematoma, and poor oxygenation at the injection site [130].

Clinical applications

In the past decade, many applications of ASCs have been developed for clinical use. In the beginning, most of these applications involved plastic surgery [154]. The use of both SVF and ASCs has tremendously increased since 2010; minimum 3000 patients have been benefiting from this kind of therapy, for more than 10 different diseases (*e.g.*, plastic surgery, diabetes mellitus, digestive, cardiovascular, autoimmune, neurological, hematological, immunological, urological or lung diseases, skeletal regeneration) [154].

Due to this enormous potential, the next step for ASCs therapy is clinical banking for both autologous and allogeneic transplants of stem cells [72]; this is an "off-the-shelf" approach that would insure the availability of stem cells in acute and chronic disorders [155]. At present, different companies provide banking of ASCs or adipose tissue, but the compliance with the Good Manufacturing Practices (GMP) guidelines related to harvesting, processing and cryopreservation is, in many cases, questionable [72].

The characteristics of ASCs, such as multipotency, immunomodulation, neo-angiogenesis, and reparatory mechanisms make them very suitable for clinical use [72]. To date, there are many undergoing ASCs-based clinical trials worldwide, European countries, Korea and Mexico being the most involved. US has only several clinical trials due to their rigorous regulation by US FDA and despite having the most extensive preclinical research and number of publications on this topic [72]. In Table 14, some of *NIH* clinical trials (in early phase or phases I–IV), that use ASCs as therapy are presented [11, 156].

Table 14 – ***ASCs clinical trials in early phase, phase I, II, III and IV*** **[11, 156]**

No.	Title	Status	Study results	Conditions	Interventions	Locations
			Top 10 (out of 45) completed			
1.	Safety and efficacy of adipose-derived stem cells for chronic obstructive pulmonary disease	Completed	No results available	COPD	Drug: ASCs therapy	US
2.	Safety and efficacy study of allogeneic adipose-derived stem cells for treatment of lateral epicondylitis	Completed	No results available	Tennis elbow	Biological: ALLO-ASC-TI Drug: placebo	Korea
3.	Safety and preliminary efficacy of adipose-derived stem cells and low frequency ultrasound in PAD	Completed	No results available	PAD	Biological: LoFU and ASCs Biological: ASCs	US
4.	Safety and efficacy study of autologous cultured adipose-derived stem cells for the Crohn's fistula	Completed	No results available	Crohn's fistula	Drug: ADIPOPLUS	Korea
5.	Study to assess the safety and efficacy of expanded allogeneic adipose-derived stem cells (eASCs) (Cx601) for treatment of complex perianal fistulas in perianal Crohn's disease	Completed	No results available	Crohn's disease Anal fistula	Drug: Cx601	Spain
6.	Safety and efficacy study of autologous cultured adipose-derived stem cells for the Crohn's fistula	Completed	No results available	Crohn's fistula	Biological: ADIPOPLUS	Korea
7.	Immunophenotyping of fresh stromal vascular fraction from adipose-derived stem cells (ASCs) enriched fat grafts	Completed	Has results	Breast reconstruction Contour irregularities Volume insufficiency	Genetic: centrifuged fat graft Genetic: ASCs enriched fat graft	Brazil
8.	Clinical trial study about human adipose-derived stem cells in the stroke	Completed	No results available	Stroke	Drug: ASCs	Taiwan
9.	Clinical trial study about human adipose-derived stem cells in the liver cirrhosis	Completed	No results available	Liver cirrhosis	Drug: ASCs	Taiwan
10.	Randomized clinical trial of adipose-derived stem cells in the treatment of patients with ST-elevation myocardial infarction	Completed	No results available	Myocardial infarction Coronary arteriosclerosis Cardiovascular disease Coronary disease	Drug: Injection of ADRCs Other: Injection of placebo	Netherlands, Spain
			Top 5 (out of 5) terminated			
11.	Clinical trials of autologous cultured adipose-derived stem cells (ANTG-ASC) on complex fistula	Terminated	No results available	Complex perianal fistula	Biological: autologous cultured ASCs (low dose group) Biological: autologous cultured ASCs (high dose group)	Korea

No.	Title	Status	Study results	Conditions	Interventions	Locations
12.	Safety study of autologous cultured adipose-derived stem cells for the fecal incontinence	Terminated	No results available	Fecal incontinence	Biological: ANT-SM	Korea
13.	Safety and efficacy of adipose-derived stem cells to treat complex perianal fistulas patients with Crohn's disease	Terminated	No results available	Complex perianal fistula Crohn's disease	Drug: eASCs Drug: placebo	Austria, Netherlands, Spain
14.	Safety and efficacy of adipose-derived stem cells in refractory rheumatoid arthritis, systemic lupus erythematosus or Sharp's syndrome	Terminated	No results available	Systemic lupus erythematosus Rheumatoid arthritis Sharp's syndrome	Other: intravenous injection of SVF cells containing ASCs Other: Lipoaspiration	US
15.	Multi-center study safety of adipose-derived mesenchymal stem cells for the treatment of multiple sclerosis	Terminated	No results available	Multiple sclerosis	Biological: autologous adipose-derived MSCs	Cayman Islands
	Top 7 (out of 7) active, not recruiting					
16.	Safety, tolerability and preliminary efficacy of adipose-derived stem cells for patients with COPD	Active not recruiting	No results available	COPD	Biological: ASCs administration	US
17.	The impact of *N*-acetylcysteine on volumetric retention of autologous fat graft for breast asymmetry correction	Active not recruiting	No results available	Congenital breast deformity Graft loss Adipose tissue atrophy Breast atrophy	Drug: NAC	Poland
18.	Clinical trial to compare ReJoin™ to Sodium Hyaluronate injection for knee osteoarthritis cartilage defects	Active not recruiting	No results available	Defect of articular cartilage KO	Biological: ReJoin™ Drug: Sodium Hyaluronate	China
19.	Follow-up study for participants jointstem clinical trial	Active not recruiting	No results available	KO	Drug: jointstem	Korea
20.	Hypoxia and inflammatory injury in human renovascular hypertension	Active not recruiting	No results available	Renal artery stenosis Ischemic nephropathy Renovascular disease CKD	Drug: MSCs Procedure: MSCs delivery with stent placement	US
21.	Intrathecal autologous ADRC treatment of autoimmune refractory epilepsy	Active not recruiting	No results available	Refractory epilepsy	Biological: ADRC transplantation in autoimmune refractory epilepsy	
22.	Stem cell fistula plug in perianal Crohn's disease	Active not recruiting	No results available	Perianal Crohn's disease	Drug: MSCs–AFP	US
	Top 10 (out of 41) recruiting					
23.	Adipose-derived stem cells *versus* platelet-rich plasma on follicular unit extraction	Recruiting	No results available	Androgenetic alopecia	Biological: ASCs suspension Biological: PRP	Egypt
24.	A clinical study using adipose-derived stem cells for diabetic foot	Recruiting	No results available	Peripheral vascular disease Ischemia Diabetic foot	Biological: ASCs Biological: saline	China
25.	Adipose-derived stem cell injections for knee osteoarthritis	Recruiting	No results available	KO Knee pain	Procedure: stem cells injection Drug: corticosteroid injection	US
26.	Adipose-derived stem cells (ASCs) for moderate to severe chronic kidney disease	Recruiting	No results available	Moderate to severe CKD	Drug: Elixcyte	Taiwan
27.	Adipose-derived stem cells (ASCs) for knee osteoarthritis	Recruiting	No results available	KO	Biological: Elixcyte 8 mL Device: Hya Joint Plus Biological: Elixcyte 4 mL Biological: Elixcyte 2 mL	Taiwan
28.	Adult allogenic expanded adipose-derived stem cells (eASCs) for the treatment of complex perianal fistula(s) in patients with Crohn's disease	Recruiting	No results available	Crohn's disease	Drug: Cx601 Other: placebo	Belgium, Czechia, France and 51 more
29.	Stem cell therapy in ischemic non-treatable cardiac disease	Recruiting	No results available	Heart failure	Biological: CSCC_ASC	Denmark
30.	^{19}F hot spot MRI of human adipose-derived stem cells for breast reconstruction	Recruiting	No results available	Breast cancer	Drug: CS-1000 labeled SVF cells	US
31.	First in humans to evaluate collagen patches with stem cells in patients with ischemic left ventricular dysfunction	Recruiting	No results available	Heart failure with reduced ejection fraction	Device: VB-C01	Spain

No.	Title	Status	Study results	Conditions	Interventions	Locations
32.	Cx611-0204 SEPCELL study	Recruiting	No results available	Bacterial pneumonia	Drug: Cx611 or placebo	Belgium, Spain and 10 more
	Top 10 (out of 10) not yet recruiting					
33.	Knee osteoarthritis treatment with adipose-derived stem cells: phase II clinical trial	Not yet recruiting	No results available	KO	Biological: stem cells	Saudi Arabia
34.	Safety of adipose-derived stem cell stromal vascular fraction	Not yet recruiting	No results available	Abnormally healing wounds Scars Soft tissue defects	Biological: ADSC-SVF-002	Canada
35.	Multicenter trial of stem cell therapy for osteoarthritis (MILES)	Not yet recruiting	No results available	Osteoarthritis	Biological: autologous BMAC Biological: adipose-derived SVF Biological: UCT Drug: Depomedrol and normal saline (corticosteroid injection)	US
36.	Evaluation of soft tissue profile changes following autogenous fat or onlay polyetheretherketone (PEEK) augmentation *versus* sliding genioplasty for correction of deficient chin	Not yet recruiting	No results available	Patients with deficient chin	Procedure: fat filler, PEEK, sliding genioplasty	
37.	Treatment of tendon disease using autologous adipose-derived mesenchymal stem cells	Not yet recruiting	No results available	Rotator cuff tear Lateral epicondylitis	Biological: autologous adipose-derived MSCs Drug: compound Betamethasone	China
38.	Stem cell therapy in non-ischemic non-treatable dilated cardiomyopathies II: a pilot study	Not yet recruiting	No results available	Non-ischemic dilated cardiomyopathy	Biological: allogeneic adipose-derived stromal cells (CSCC_ASC) Other: control group	Denmark
39.	Safety study of Stemchymal® in acute liver failure	Not yet recruiting	No results available	Stem cells Adult stem cells Acute liver failure Acute-on-chronic liver failure Steminent	Biological: Stemchymal®	Taiwan
40.	Assessment of the efficacy and tolerance of sub-cutaneous re-injection of autologous adipose-derived regenerative cells in the local treatment of neuropathic diabetic foot ulcers	Not yet recruiting	No results available	Diabetic foot ulcer	Drug: ADRCs	France
41.	Scleroderma and adipose-derived stromal cells	Not yet recruiting	No results available	Systemic sclerosis	Drug: SVF Drug: Ringer lactate	France
42.	Study to assess the safety and efficacy of an IT administration of SCM-010 in SPMS	Not yet recruiting	No results available	SPMS	Biological: SCM-010	Israel

ASCs: Adipose-derived stem cells; PAD: Peripheral arterial disease; COPD: Chronic obstructive pulmonary disease; ADRC: Adipose-derived regenerative cell; MRI: Magnetic resonance imaging; PEEK: Polyetheretherketone; IT: Intrathecal; SPMS: Secondary-progressive multiple sclerosis; KO: Knee osteoarthritis; CKD: Chronic kidney disease; LoFU: Low frequency ultrasound; SVF: Stromal vascular fraction; MSCs: Mesenchymal stem cells; NAC: *N*-acetylcysteine; AFP: Alpha-fetoprotein; PRP: Platelet-rich plasma; CSCC: Cardiology Stem Cell Centre; BMAC: Bone marrow aspirate concentrate; UCT: Umbilical cord tissue.

The most important clinical applications are presented below.

Bone and cartilage

ASCs have a potential benefit for skeletal defects [72]. One of the first reports of the use of ASCs in bone regeneration belongs to Lendeckel *et al.*, involving calvarial reconstruction in a young patient with severe skull fractures [157]. Thesleff *et al.* also reported skull regeneration in four patients with cranial defects [158]. Mesimäki *et al.* combined the use of ASCs, beta-tricalcium phosphate (β-TCP) and BMP-2 for the regeneration of a resected palate in an older patient [159]. Later, Gimble *et al.* used the same approach in 20 patients with maxillo-facial defects [160]. Pak reported the use of autologous SVF for the treatment of femoral head necrosis [161] and the use of ASCs in the treatment of osteoarthritis [162].

Cardiovascular disease

Many studies reported that ASCs have a potential for heart regeneration in acute and chronic myocardial infarction in animal models [163–166]. Due to these reports, human clinical trials have started on patients with both acute and chronic myocardial infarction [72, 167]. Based on their capacity of neo-angiogenesis, anti-apoptosis, and transdifferentiation into endothelial cells and pericytes, ASCs are also currently studied for the treatment of peripheral vascular disease [72, 129, 168–172].

Soft tissue augmentation and reconstructive surgery

The use of autologous fat grafts for facial rejuvenation and soft tissue augmentation is well known [173]; however, in many cases the results of these procedures are variable and unpredictable, due to the possible necrosis, fibrosis or fat resorption [173].

A novel technique with better effects than simple autologous fat grafting was developed to overcome these problems. It refers to the conversion of aspirated fat into an ASC-rich adipose tissue by adding ASCs [72–75, 174, 175]. Both CAL and stem cell-enriched tissue (SET) injections procedures are based on this principle. SET involves the injection of autologous ASCs in an area that was previously (hours before) injected with autologous fat [174, 175].

Mature adipocytes obtained from autologous ASCs are used for the treatment of patients with depressed scars [176]. ASCs appear to be also beneficial in wound healing due to their capacity of differentiation into dermal fibroblasts and keratinocytes, and the trophic support in the regenerating tissue [177–182]. Some studies reported the beneficial effects of SVF for the treatment of radiation wounds [183, 184], while others reported the use of autologous fat grafts in treating ulcerative, dystrophic or radiation wounds [185, 186].

Immune disease

ASCs immunomodulation features make them valuable for the treatment of immunological disorders [187, 188]. Some studies reported the use of ASC in the treatment of graft *vs.* host disease [189, 190] and hematological diseases resulting from ABO-incompatible transplantations [191–195].

ASCs use was also reported in fistula repair [196]. Comparing the use of expanded ASCs *vs.* freshly-isolated SVF in the fistula therapy in Crohn's disease, Garcia-Olmo *et al.* revealed that expanded ASCs provide better results [197]; these are probably due to the differences in immunomodulation, since ASCs suppress T-cell proliferation, while SVF stimulates T-cell proliferation [72, 197, 198].

Diabetes mellitus

ASCs could be potentially used for insulin replacement therapy, since it was proved that patients, who received an infusion of cultured BMSCs and ASCs insulin-secreting cells, presented less exogenous need of insulin, decreased risk for ketoacidosis and decreased hemoglobin A1c (HbA1c) levels [199].

Neural disorders

Studies suggest that ASCs can differentiate into neuronal-like stem cells like BMSCs, but with a higher proliferation capacity [200]. Recent research has focused on Parkinson's and Alzheimer's diseases or spinal cord injuries, where ASCs characteristics, such as migration and differentiation into neurons, bystander effects of cytokines, and activation of native microglia, could have beneficial outcomes [41, 146, 201].

Esthetic surgery

ASCs use in facial rejuvenation includes the correction of dark circles, hollow eyes, malar bags or adjuvant blepharoplasty. Traditional fat grafting or new techniques (*e.g.*, micro-, ultra-micro-, or nanografting) are used [31, 137, 202–204]. Fat transplant can also be used in hand rejuvenation [36, 205]. In augmentation rhinoplasty, autologous fat grafting is very useful compared to synthetic implants [206].

Other disorders

ASCs are currently under research for other diseases as pulmonary fibrosis [207] and muscular dystrophy [208–210].

Since ASCs represent such a promising tool for clinical use, any possible drawbacks need to be very well assessed before procedures to be introduced into daily practice [72].

Barriers in the knowledge process

The efficacy of ASCs therapies can be influenced by a multitude of factors, primarily related to the biological properties of the cells, the technical challenges and the non-standard protocols. The previous registered heterogeneous results of ASCs therapeutic efficacy impaired the comparisons between clinical trials [41].

Therefore, it is important to asses unifying criteria regarding: the internal parameters (*e.g.*, reagents sources, enzyme qualities, serum alternatives, closed system devices use, contamination testing, donors variability), the external ones (*e.g.*, shipping) [5] and the operating procedures (*e.g.*, selection of the most appropriate and resistant ASCs lines, best site of grafting, transplanted cells adequate number, transplanted ASCs and microenvironment compatibility, cell isolation and culture methods) [11]; moreover, tracking the implanted cells is vital to their targeted migration, survival and differentiation [41].

Considerable concern is rising about the relationship between ASCs and tumorigenesis [1, 4–6, 8, 72, 211, 212]; the ability of ASCs to promote residual tumor cells to proliferate, differentiate and metastasize or, to induce the occurrence of *de novo* neoplasms is still under debate [1].

It was shown that *in vitro* human ASCs can undergo malignant transformation with passaging over four months [40]; as a result, investigators favor the use of freshly isolated ASCs (safer and more practical in clinical applications) *vs.* cultured ones [40]. It was also demonstrated that in cancer reconstructions, ASCs used for tissue regeneration, can stimulate the tumor growth. Moreover, ASCs conditioned medium processed from patients with cancer produces more paracrine factors (*e.g.*, VEGF), than the one from cancer-free controls; the ASCs increased secretion of chemokines that might accelerate motility, invasion and metastasis of residual cancer cells is involved in both cases [1]. It seems too, that, ASCs express tumor tropism and thus, the engraftment of cells in the neoplastic mass enhance the development of a permissive fibrovascular stroma that facilitates tumor growth and metastasis [14]. Furthermore, by immunofluorescence, an exosomal vesicles transfer was highlighted between ASCs and tumor cells.

Accordingly, even if ASCs do not form tumors by themselves, they favor the growth of pre-existing ones and therefore the treatment with stem cells in cancer patients is not advised [1]. On the other hand, consistent with the data obtained from RESTORE-2 clinical trial about the role of ASCs in reconstruction after breast cancer, if the patient is clinically disease free, the ASCs therapy seems to be safe [39].

If literature provides numerous studies that reveal the participation of ASCs in tumor progression, there are papers that describe the antitumor effect of ASCs. In relation to a certain type of cancer, many factors may be implicated: the secretion of tumor suppression chemokines [*e.g.*, Dickkopf WNT signaling pathway inhibitor 1 (DKK1) inhibitor of β-catenin pathway, in chronic myelogenous leukemia], apoptosis [*e.g.*, *via* poly adenosine diphosphate (ADP) ribose polymerase cleavage and production of caspase 3, in lung cancer], tumor cells necrosis and death [*e.g.*, down-regulation of cyclin-dependent kinase 4 (CDK4) with G1 phase arrest, in pancreatic adenocarcinoma ductal cells]. Engineered ASCs with coadministration of a chemotherapeutic drug can also create an immunosuppressive environment for tumors [11, 14].

A dual effect of ASCs that can simultaneous promote and inhibe the tumor growth, is apparently due to the heterogeneous reactions that these cells exert on host environment [14].

Another big concern is the use of culture-expanded ASCs that might bring additional risk into therapy, due to their ability to escape senescence [213, 214]; however, the studies are still controversial [72, 215].

Regulatory aspects

Due to potential secondary side effects, the use of stem cells in regenerative medicine has to fulfill some requirements [1], having in mind the safety of the patient and the clinical efficiency [1].

Different countries offer different guidelines and regulations, which sometimes are not identical [1], this resulting in an economical impact that favors the most flexible countries [1]. Three largest pharmaceutical markets, US, Asia (Japan) and Europe, are the international leaders on the biological products and related devices, and are responsible for the most rigorous approval guidelines [5].

In the US, the major jurisdiction over stromal/stem cell products is the *Center for Biologics Evaluation and Research* (CBER), section of the US *FDA* [5]. According to US *FDA* [216], the definition for ASCs used in clinical practice is: human cells/tissues or cellular/tissue-based products, intended either for implantation, infusion or transfer into a human recipient (title 21 code of federal regulations part 11271) or as biological drug (section 351 of the public health services act), this depending on the specific use and degree of processing [39, 216].

In Europe, under the supervision of *European Medicines Agency* (EMA), which acts as a centralized regulatory agency for EU members states, therapeutic products, such as those based on gene therapy, somatic cell therapy or tissue engineering are defined as advanced therapy medicinal products (ATMP) and fall under the supervision of European Commission (EC) Regulation 1394/2007 Guidelines, effective since December 2008 [1]. These guidelines, stated by EMA or the other national authorities (in Asia – Japan, the *Pharmaceutical and Medical Devices Agency*) [5], cover specific rules for GMP and Good Clinical Practice (GCP), as well as specific rules for labeling, packaging and traceability, efficacy, adverse reactions and risk management [1, 5]; they were established to ensure that patients are not at risk and the therapy is safe and efficient [5].

Cells or tissues that are considered to be engineered have to fulfill minimum one of the following criteria: to be subjected to substantial manipulation and/or to be used for different essential functions (*e.g.*, non-homologous use) [40].

Cell culturing and enzymatic digestion are considered substantial manipulation, so they fall under the ATMP guidelines. In view of this, collagenase digestion for the isolation of ASCs is considered substantial manipulation [40].

Contrary, mechanical isolation (from subcutaneous adipose tissue of cryopreserved ASCs or of adult, viable, autologous, unexpanded or uncultured ASCs from SVF suspensions) is not included under this definition [40]. In this case, no regulation needs to be followed and no laws apply, because the cells have been minimally manipulated [40]; they can be used only for regeneration, repair, and replacement of weakened or injured subcutaneous adipose tissue [2, 40, 217] (EMA/129056/2013). Since 2015, the Committee for Advanced Therapies (CAT) stated the use of collagenase digestion for the isolation of ASCs as substantial manipulation and therefore advanced therapy [218]; each case where enzymatic digestion is part of the isolation protocol is evaluated on a case-by-case basis, depending on the type of tissue [40, 217]. On the other hand, ASCs to be used in other location than subcutaneous tissue are also considered ATMP.

In Europe, closed mechanical devices for SVF cells isolation using collagenase digestion are approved and this has facilitated the research and point-of-care delivery for clinicians. In US, they are forbidden and regulated as class III medical devices, used only as research tools [5].

In conclusion, in order to follow the consecrated regulations, ASCs to be used in patients should be: autologous, minimally manipulated, derived from subcutaneous tissue, reinjected during the same surgical act and not belonging to the category: autologous/allogeneic, culture-expanded cells [14].

An international unifying concept regarding cell therapies policies will be beneficial, due to their continuous updating, revision and development in order to maintain the level of the last scientific findings [14].

Conclusions

Presently, the ongoing research and the significant advances point out to the tremendous potential of using ASCs for reconstructive medicine. These cells represent a therapeutic field in continuous development. Due to the rapid accumulation of scientific information, it allowed for the first time in the history of stem cells concept, successful translational movements from bench-to-bedside. If the classic therapeutic experimental design of stem cells paradigm “hypothesis-driven, mechanism-based” was not

always respected, all international scientific efforts on ASCs testing and regulation focused primarily on patient safety and product efficacy. The existing drawbacks like the local trauma in the harvest area, the significant reduction in the adipose graft volume, the low cell survival rate, the poor engraftment in the target tissue, the entire non-standard processing techniques, the restrictive legislation, or the still uncertain clinical strategies, are future challenges, with optimism researched further, until all gaps in knowledge can be completed.

Conflict of interests

The authors state that there is no known conflict of interests associated with this publication.

References

[1] Frese L, Dijkman PE, Hoerstrup SP. Adipose tissue-derived stem cells in regenerative medicine. Transfus Med Hemother, 2016, 43(4):268–274.

[2] Simonacci F, Bertozzi N, Raposio E. Off-label use of adipose-derived stem cells. Ann Med Surg (Lond), 2017, 24:44–51.

[3] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell, 2002, 13(12):4279–4295.

[4] Bellini E, Grieco MP, Raposio E. The science behind autologous fat grafting. Ann Med Surg (Lond), 2017, 24:65–73.

[5] Gimble JM, Bunnel BA, Guilak F. Human adipose-derived cells: an update on the transition to clinical translation. Regen Med, 2012, 7(2):225–235.

[6] Kokai LE, Marra K, Rubin JP. Adipose stem cells: biology and clinical applications for tissue repair and regeneration. Transl Res, 2014, 163(4):399–408.

[7] Simonacci F, Bertozzi N, Grieco MP, Grignaffini E, Raposio E. Procedure, applications, and outcomes of autologous fat grafting. Ann Med Surg (Lond), 2017, 20:49–60.

[8] Mizuno H. Adipose-derived stem cells for regenerative medicine in the field of plastic and reconstructive surgery. J Oral Biosci, 2013, 55(3):132–136.

[9] Raposio E, Simonacci F, Perrota RE. Adipose-derived stem cells: comparison between two methods of isolation for clinical applications. Ann Med Surg (Lond), 2017, 20:87–91.

[10] Nae S, Bordeianu I, Stăncioiu AT, Antohi N. Human adipose-derived stem cells: definition, isolation, tissue-engineering applications. Rom J Morphol Embryol, 2013, 54(4):919–924.

[11] Argentati C, Morena F, Bazzucchi M, Armentano I, Emiliani C, Martino S. Adipose stem cell translational applications: from bench-to-bedside. Int J Mol Sci, 2018, 19(11):3475.

[12] Ong WK, Sugii S. Adipose-derived stem cells: fatty potentials for therapy. Int J Biochem Cell Biol, 2013, 45(6):1083–1086.

[13] Zuk P. Adipose derived stem cells in tissue regeneration: a review. ISRN Stem Cells, 2013, 2013:713959.

[14] Kapur SK, Dos-Anjos Vilaboa S, Llull R, Katz AJ. Adipose tissue and stem/progenitor cells: discovery and development. Clin Plast Surg, 2015, 42(2):155–167.

[15] Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg, 1989, 83(2):368–381.

[16] Uroskie TW, Colen LB. History of breast reconstruction. Semin Plast Surg, 2004, 18(2):65–69.

[17] Patel BC, Malhotra R. Mid forehead brow lift. StatPearls [Internet], StatPearls Publishing, Treasure Island, FL, 2018 Dec 9, available at: https://www.ncbi.nlm.nih.gov/books/NBK535452.

[18] Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. Dermatol Surg, 2000, 26(12):1159–1166.

[19] Obi LJ. Specialized stem cell fat transfer to face. In: Shiffman MA, Di Giuseppe A, Bassetto F (eds). Stem cells in aesthetic procedures. Springer-Verlag, Berlin–Heidelberg, 2014, 441–461.

[20] Nelson L, Stewart KJ. Autologous fat transfer for HIV-associated facial lipodystrophy. In: Erian A, Shiffman MA (eds). Advanced surgical facial rejuvenation: art and clinical practice. Springer-Verlag, Berlin–Heidelberg, 2012, 707–721.

[21] Gejje S, Singh AK, Srivastava PK, Goel M, Kumar V, Hongal A. Evaluation of use of adipose cells in various procedures of plastic surgery and its volumetric quantification using high resolution ultrasound. J Clin Diagn Res, 2017, 11(8):PC17–PC21.

[22] Ross RJ, Shayan R, Mutimer KL, Ashton MW. Autologous fat grafting: current state of the art and critical review. Ann Plast Surg, 2014, 73(3):352–357.

[23] Mojallal A, Foyatier JL. Historique de l'utilisation du tissu adipeux comme produit de comblement en chirurgie plastique. Ann Chir Plast Esthet, 2004, 49(5):419–425.

[24] Zielins ER, Brett EA, Longaker MT, Wan DC. Autologous fat grafting: the science behind the surgery. Aesthet Surg J, 2016, 36(4):488–496.

[25] Dhami LD. Liposuction. Indian J Plast Surg, 2008, 41(Suppl): S27–S40.

[26] Toledo LS. Principles of autologous fat transplantation. In: Illouz YG, Sterodimas A (eds). Adipose stem cells and regenerative medicine. Springer-Verlag, Berlin–Heidelberg, 2011, 45–46.

[27] Illouz YG. Body contouring by lipolysis: a 5-year experience with over 3000 cases. Plast Reconstr Surg, 1983, 72(5):591–597.

[28] Fournier PF, Otteni FM. Lipodissection in body sculpturing: the dry procedure. Plast Reconstr Surg, 1983, 72(5):598–609.

[29] Illouz YG. Illouz's technique of body contouring by lipolysis. Clin Plast Surg, 1984, 11(3):409–417.

[30] Smahel J. Experimental implantation of adipose tissue fragments. Br J Plast Surg, 1989, 42(2):207–211.

[31] Coleman SR. Facial recontouring with lipostructure. Clin Plast Surg, 1997, 24(2):347–367.

[32] Ersek RA. Transplantation of purified autologous fat: a 3-year follow-up is disappointing. Plast Reconstr Surg, 1991, 87(2): 219–227; discussion 228.

[33] Carpaneda CA, Ribeiro MT. Percentage of graft viability *versus* injected volume in adipose autotransplants. Aesthetic Plast Surg, 1994, 18(1):17–19.

[34] Pu LLQ, Cui X, Fink BF, Cibull ML, Gao D. Long-term preservation of adipose aspirates after conventional lipoplasty. Aesthet Surg J, 2004, 24(6):536–541.

[35] Pu LL, Cui X, Fink BF, Cibull ML, Gao D. The viability of fatty tissues within adipose aspirates after conventional liposuction: a comprehensive study. Ann Plast Surg, 2005, 54(3):288–292; discussion 292.

[36] Coleman SR. Facial augmentation with structural fat grafting. Clin Plast Surg, 2006, 33(4):567–577.

[37] Yang H, Lee H. Successful use of squeezed-fat to correct a breast affected by Poland syndrome. Aesthetic Plast Surg, 2011, 35(3):418–425.

[38] Sterodimas A, de Faria J, Nicaretta B, Pitanguy I. Tissue engineering with adipose-derived stem cells (ADSCs): current and future applications. J Plast Reconstr Aesthet Surg, 2010, 63(11):1886–1892.

[39] Minteer DM, Marra KG, Rubin JP. Adipose stem cells: biology, safety, regulation, and regenerative potential. Clin Plast Surg, 2015, 42(2):169–179.

[40] Raposio E, Ciliberti RG. Clinical use of adipose-derived stem cells: European legislative issues. Ann Med Surg (Lond), 2017, 24:61–64.

[41] Feisst V, Meidinger S, Locke MB. From bench to bedside: use of human adipose-derived stem cells. Stem Cell Cloning, 2015, 8:149–162.

[42] Gimble JM, Bunnel BA, Chiu ES, Guilak F. Concise review: Adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation. Stem Cells, 2011, 29(5):749–754.

[43] Salgado AJ, Reis RL, Sousa NJ, Gimble JM. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. Curr Stem Cell Res Ther, 2010, 5(2):103–110.

[44] von Heimburg D, Zachariah S, Low A, Pallua N. Influence of different biodegradable carriers on the *in vivo* behavior of human adipose precursor cells. Plast Reconstr Surg, 2001, 108(2):411–420; discussion 421–422.

[45] Tremolada C, Palmieri G, Ricordi C. Adipocyte transplantation and stem cells: plastic surgery meets regenerative medicine. Cell Transplant, 2010, 19(10):1217–1223.

[46] Eto H, Kato H, Suga H, Aoi N, Doi K, Kuno S, Yoshimura K. The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. Plast Reconstr Surg, 2012, 129(5):1081–1092.
[47] Yang HJ, Kim KJ, Kim MK, Lee SJ, Ryu YH, Seo BF, Oh DY, Ahn ST, Lee HY, Rhie JW. The stem cell potential and multipotency of human adipose tissue-derived stem cells vary by cell donor and are different from those of other types of stem cells. Cells Tissues Organs, 2014, 199(5–6):373–383.
[48] Schipper BM, Marra KG, Zhang W, Donnenberg AD, Rubin JP. Regional anatomic and age effects on cell function of human adipose-derived stem cells. Ann Plast Surg, 2008, 60(5): 538–544.
[49] Xu Y, Malladi P, Wagner DR, Longaker MT. Adipose-derived mesenchymal cells as a potential cell source for skeletal regeneration. Curr Opin Mol Ther, 2005, 7(4):300–305.
[50] Aksu AE, Rubin JP, Dudas JR, Marra KG. Role of gender and anatomical region on induction of osteogenic differentiation of human adipose-derived stem cells. Ann Plast Surg, 2008, 60(3):306–322.
[51] Morena F, Argentati C, Calzoni E, Cordellini M, Emiliani C, D'Angelo F, Martino S. *Ex-vivo* tissues engineering modeling for reconstructive surgery using human adult adipose stem cells and polymeric nanostructured matrix. Nanomaterials (Basel), 2016, 6(4):57.
[52] van Harmelen V, Skurk T, Röhrig K, Lee YM, Halbleib M, Aprath-Husmann I, Hauner H. Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women. Int J Obes Relat Metab Disord, 2003, 27(8):889–895.
[53] Gentile P, Orlandi A, Scioli MG, Di Pasquali C, Bocchini I, Cervelli V. Concise review: Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. Stem Cells Transl Med, 2012, 1(3):230–236.
[54] Levi B, James AW, Glotzbach JP, Wan DC, Commons GW, Longaker MT. Depot-specific variation in the osteogenic and adipogenic potential of human adipose-derived stromal cells. Plast Reconstr Surg, 2010, 126(3):822–834.
[55] Dragoo JL, Chang W. Arthroscopic harvest of adipose-derived mesenchymal stem cells from the infrapatellar pad. Am J Sports Med, 2017, 45(13):3119–3127.
[56] SundarRaj S, Deshmukh A, Priya N, Krishnan VS, Cherat M, Majumdar AS. Development of a system and method for automated isolation of stromal vascular fraction from adipose tissue lipoaspirate. Stem Cells Int, 2015, 2015:109353.
[57] Fraser JK, Hicok KC, Shanahan R, Zhu M, Miller S, Arm DM. The Celution® system: automated processing of adipose-derived regenerative cells in a functionally closed system. Adv Wound Care (New Rochelle), 2014, 3(1):38–45.
[58] Hicok KC, Hedrick MH. Automated isolation and processing of adipose-derived stem and regenerative cells. In: Gimble JM, Bunnell BA (eds.) Adipose-derived stem cells: methods and protocols. Vol. 702, Series "Methods in Molecular Biology", Humana Press–Springer, New York, 2011, 87–105.
[59] Aronowitz JA, Ellenhorn JD. Adipose stromal vascular fraction isolation: a head-to-head comparison of four commercial cell separation systems. Plast Reconstr Surg, 2013, 132(6):932e–939e.
[60] Aronowitz JA. Reply: Adipose stromal vascular fraction isolation: a head-to-head comparison of four commercial cell separation systems. Plast Reconstr Surg, 2014, 133(6):889e–890e.
[61] Aronowitz JA, Lockhart RA, Hakakian CS. Mechanical *versus* enzymatic isolation of stromal vascular fraction cells from adipose tissue. SpringerPlus, 2015, 4:713.
[62] Wang L, Lu Y, Luo X, Fu MG, Hu X, Dong H, Fan ZH. [Cell-assisted lipotransfer for breast augmentation: a report of 18 patients]. Zhonghua Zheng Xing Wai Ke Za Zhi, 2012, 28(1):1–6.
[63] Brown JC, Shang H, Li Y, Yang N, Patel N, Katz AJ. Isolation of adipose-derived stromal vascular fraction cells using a novel point-of-care device: cell characterization and review of the literature. Tissue Eng Part C Methods, 2017, 23(3):125–135.
[64] Dos-Anjos Vilaboa S, Navarro-Palou M, Llull R. Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates. Cytotherapy, 2014, 16(8):1092–1097.
[65] Oberbauer E, Steffenhagen C, Wurzer C, Gabriel C, Redl H, Wolbank S. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. Cell Regen (Lond), 2015, 4:7.
[66] Pak J, Lee JH, Lee SH. Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells. Biomed Res Int, 2014, 2014:436029.
[67] Bora P, Majumdar AS. Adipose tissue-derived stromal vascular fraction in regenerative medicine: a brief review on biology and translation. Stem Cell Res Ther, 2017, 8(1):145.
[68] Güven S, Karagianni M, Schwalbe M, Schreiner S, Farhadi J, Bula S, Bieback K, Martin I, Scherberich A. Validation of an automated procedure to isolate human adipose tissue-derived cells by using the Sepax® technology. Tissue Eng Part C Methods, 2012, 18(8):575–582.
[69] Doi K, Tanaka S, Iida H, Eto H, Kato H, Aoi N, Kuno S, Hirohi T, Yoshimura K. Stromal vascular fraction isolated from lipoaspirates using an automated processing system: bench and bed analysis. J Tissue Eng Regen Med, 2013, 7(11):864–870.
[70] Williams SK, Kosnik PE, Kleiner LB, Vossman EM, Lye KD, Shine MH. Adipose stromal vascular fraction cells isolated using an automated point of care system improve the patency of expanded polytetrafluoroethylene vascular grafts. Tissue Eng Part A, 2013, 19(11–12):1295–1302.
[71] Millan A, Landerholm T, Chapman JR. Comparison between collagenase adipose digestion and StromaCell mechanical dissociation for mesenchymal stem cell separation. McNair Scholars J CSUS, 2014, 15:86–101.
[72] Lim MH, Ong WK, Sugii S. The current landscape of adipose-derived stem cells in clinical applications. Expert Rev Mol Med, 2014, 16:e8.
[73] Sterodimas A, de Faria J, Nicaretta B, Papadopoulos O, Papalambros E, Illouz YG. Cell-assisted lipotransfer. Aesthet Surg J, 2010, 30(1):78–81.
[74] Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, Aiba-Kojima E, Iizuka F, Inoue K, Suga H, Yoshimura K. Cell-assisted lipotransfer: supportive use of human adipose derived cells for soft tissue augmentation with lipoinjection. Tissue Eng, 2006, 12(12):3375–3382.
[75] Sterodimas A, de Faria J, Nicaretta B, Boriani F. Autologous fat transplantation *versus* adipose-derived stem cell-enriched lipografts: a study. Aesthet Surg J, 2011, 31(6):682–693.
[76] Jeon BG, Kumar BM, Kang EJ, Ock SA, Lee SL, Kwack DO, Byun JH, Park BW, Rho GJ. Characterization and comparison of telomere length, telomerase and reverse transcriptase activity and gene expression in human mesenchymal stem cells and cancer cells of various origins. Cell Tissue Res, 2011, 345(1):149–161.
[77] Zuk PA. The adipose-derived stem cell: looking back and looking ahead. Mol Biol Cell, 2010, 21(11):1783–1787.
[78] Wang C, Cen L, Yin S, Liu Q, Liu W, Cao Y, Cui L. A small diameter elastic blood vessel wall prepared under pulsatile conditions from polyglycolic acid mesh and smooth muscle cells differentiated from adipose-derived stem cells. Biomaterials, 2010, 31(4):621–630.
[79] Heydarkhan-Hagvall S, Schenke-Layland K, Yang JQ, Heydarkhan S, Xu Y, Zuk PA, MacLellan WR, Beygui RE. Human adipose stem cells: a potential cell source for cardiovascular tissue engineering. Cells Tissues Organs, 2008, 187(4):263–274.
[80] Meyer J, Salamon A, Mispagel S, Kamp G, Peters K. Energy metabolic capacities of human adipose-derived mesenchymal stromal cells *in vitro* and their adaptations in osteogenic and adipogenic differentiation. Exp Cell Res, 2018, 370(2):632–642.
[81] Grottkau BE, Lin Y. Osteogenesis of adipose-derived stem cells. Bone Res, 2013, 1(2):133–145.
[82] Stromps JP, Paul NE, Rath B, Nourbakhsh M, Bernhagen J, Pallua N. Chondrogenic differentiation of human adipose-derived stem cells: a new path in articular cartilage defect management? Biomed Res Int, 2014, 2014:740926.
[83] Mizuno H, Zuk PA, Zhu M, Lorenz HP, Benhaim P, Hedrick MH. Myogenic differentiation by human processed lipoaspirate cells. Plast Reconstr Surg, 2002, 109(1):199–209; discussion 210–211.
[84] Deshpande RS, Grayson WL, Spector AA. A modeling insight into adipose-derived stem cell myogenesis. PLoS One, 2015, 10(9):e0137918.
[85] Safford KM, Hicok KC, Safford SD, Halvorsen YDC, Wilkison WO, Gimble JM, Rice HE. Neurogenic differentiation of murine and human adipose-derived stromal cells. Biochem Biophys Res Commun, 2002, 294(2):371–379.

[86] Sugii S, Kida Y, Kawamura T, Suzuki J, Vassena R, Yin YQ, Lutz MK, Berggren WT, Izpisúa Belmonte JC, Evans RM. Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. Proc Natl Acad Sci U S A, 2010, 107(8):3558–3563.
[87] McIntosh KR, Lopez MJ, Borneman JN, Spencer ND, Anderson PA, Gimble JM. Immunogenicity of allogeneic adipose-derived stem cells in a rat spinal fusion model. Tissue Eng Part A, 2009, 15(9):2677–2686.
[88] Katz AJ, Mericli AF. Stem cells derived from fat. In: Atala A, Lanza R, Thomson JA, Nerem R (eds). Principles of regenerative medicine. 2nd edition, Academic Press, San Diego, 2011, 365–381.
[89] Cho KS, Park HK, Park HY, Jung JS, Jeon SG, Kim YK, Roh HJ. IFATS collection: immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model. Stem Cells, 2009, 27(1):259–265.
[90] Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev, 2011, 20(8): 1297–1308.
[91] Prockop DJ, Olson SD. Clinical trials with adult stem/ progenitor cells for tissue repair: let's not overlook some essential precautions. Blood, 2007, 109(8):3147–3151.
[92] Kimura Y, Ozeki M, Inamoto T, Tabata Y. Time course of *de novo* adipogenesis in Matrigel by gelatin microspheres incorporating basic fibroblast growth factor. Tissue Eng, 2002, 8(4):603–613.
[93] Masuda T, Furue M, Matsuda T. Novel strategy for soft tissue augmentation based on transplantation of fragmented omentum and preadipocytes. Tissue Eng, 2004, 10(11–12):1672–1683.
[94] Patrick CW Jr, Zheng B, Johnston C, Reece GP. Long-term implantation of preadipocyte-seeded PLGA scaffolds. Tissue Eng, 2002, 8(2):283–293.
[95] Halbleib M, Skurk T, de Luca C, von Heimburg D, Hauner H. Tissue engineering of white adipose tissue using hyaluronic acid-based scaffolds. I: *In vitro* differentiation of human adipocyte precursor cells on scaffolds. Biomaterials, 2003, 24(18):3125–3132.
[96] Kim JS, Choi JS, Cho YW. Cell-free hydrogel system based on a tissue-specific extracellular matrix for *in situ* adipose tissue regeneration. ACS Appl Mater Interfaces, 2017, 9(10):8581–8588.
[97] Osinga R, Di Maggio N, Todorov A, Allafi N, Barbero A, Laurent F, Schaefer DJ, Martin I, Scherberich A. Generation of a bone organ by human adipose-derived stromal cells through endochondral ossification. Stem Cells Transl Med, 2016, 5(8):1090–1097.
[98] Zhang S, Ma B, Liu F, Duan J, Wang S, Qiu J, Li D, Sang Y, Liu C, Liu D, Liu H. Polylactic acid nanopillar array-driven osteogenic differentiation of human adipose-derived stem cells determined by pillar diameter. Nano Lett, 2018, 18(4): 2243–2253.
[99] Kim YJ, Park SG, Shin B, Kim J, Kim SW, Choo OS, Yin XY, Min BH, Choung YH. Osteogenesis for postoperative temporal bone defects using human ear adipose-derived stromal cells and tissue engineering: an animal model study. J Biomed Mater Res A, 2017, 105(12):3493–3501.
[100] Yang G, Rothrauff BB, Lin H, Yu S, Tuan RS. Tendon-derived extracellular matrix enhances transforming growth factor-*β*3-induced tenogenic differentiation of human adipose-derived stem cells. Tissue Eng Part A, 2017, 23(3–4):166–176.
[101] Wu SC, Chen CH, Wang JY, Lin YS, Chang JK, Ho ML. Hyaluronan size alters chondrogenesis of adipose-derived stem cells *via* the CD44/ERK/SOX-9 pathway. Acta Biomater, 2018, 66:224–237.
[102] Han IH, Sun F, Choi YJ, Zou F, Nam KH, Cho WH, Choi BK, Song GS, Koh K, Lee J. Cultures of Schwann-like cells differentiated from adipose-derived stem cells on PDMS/ MWNT sheets as a scaffold for peripheral nerve regeneration. J Biomed Mater Res A, 2015, 103(11):3642–3648.
[103] Sowa Y, Kishida T, Imura T, Numajiri T, Nishino K, Tabata Y, Mazda O. Adipose-derived stem cells promote peripheral nerve regeneration *in vivo* without differentiation into Schwann-like lineage. Plast Reconstr Surg, 2016, 137(2):318e–330e.
[104] Gorecka A, Salemi S, Haralampieva D, Moalli F, Stroka D, Candinas D, Eberli D, Brügger L. Autologous transplantation of adipose-derived stem cells improves functional recovery of skeletal muscle without direct participation in new myofiber formation. Stem Cell Res Ther, 2018, 9(1):195.
[105] Björninen M, Gilmore K, Pelto J, Seppänen-Kaijansinkko R, Kellomäki M, Miettinen S, Wallace G, Grijpma D, Haimi S. Electrically stimulated adipose stem cells on polypyrrole-coated scaffolds for smooth muscle tissue engineering. Ann Biomed Eng, 2017, 45(4):1015–1026.
[106] Yong KW, Li Y, Liu F, Gao B, Lu TJ, Wan Abas WAB, Wan Safwani WKZ, Pingguan-Murphy B, Ma Y, Xu F, Huang G. Paracrine effects of adipose-derived stem cells on matrix stiffness-induced cardiac myofibroblast differentiation *via* angiotensin II type 1 receptor and Smad7. Sci Rep, 2016, 6:33067.
[107] Kook YM, Kim H, Kim S, Heo CY, Park MH, Lee K, Koh WG. Promotion of vascular morphogenesis of endothelial cells co-cultured with human adipose-derived mesenchymal stem cells using polycaprolactone/gelatin nanofibrous scaffolds. Nanomaterials (Basel), 2018, 8(2):117.
[108] Enderami SE, Soleimani M, Mortazavi Y, Nadri S, Salimi A. Generation of insulin-producing cells from human adipose-derived mesenchymal stem cells on PVA scaffold by optimized differentiation protocol. J Cell Physiol, 2018, 233(5):4327–4337.
[109] Mamidi MK, Nathan KG, Singh G, Thrichelvam ST, Mohd Yusof NAN, Fakharuzi NA, Zakaria Z, Bhonde R, Das AK, Majumdar AS. Comparative cellular and molecular analyses of pooled bone marrow multipotent mesenchymal stromal cells during continuous passaging and after successive cryopreservation. J Cell Biochem, 2012, 113(10):3153–3164.
[110] Roato I, Alotto D, Belisario DC, Casarin S, Fumagalli M, Cambieri I, Piana R, Stella M, Ferracini R, Castagnoli C. Adipose derived-mesenchymal stem cells viability and differentiating features for orthopaedic reparative applications: banking of adipose tissue. Stem Cells Int, 2016, 2016: 4968724.
[111] Devitt SM, Carter CM, Dierov R, Weiss S, Gersch RP, Percec I. Successful isolation of viable adipose-derived stem cells from human adipose tissue subject to long-term cryopreservation: positive implications for adult stem cell-based therapeutics in patients of advanced age. Stem Cells Int, 2015, 2015:146421.
[112] Harris DT. Banking of adipose- and cord tissue-derived stem cells: technical and regulatory issues. Adv Exp Med Biol, 2016, 951:147–154.
[113] Liseth K, Abrahamsen JF, Bjørsvik S, Grøttebø K, Bruserud Ø. The viability of cryopreserved PBPC depends on the DMSO concentration and the concentration of nucleated cells in the graft. Cytotherapy, 2005, 7(4):328–333.
[114] Benekli M, Anderson B, Wentling D, Bernstein S, Czuczman M, McCarthy P. Severe respiratory depression after dimethyl-sulphoxide-containing autologous stem cell infusion in a patient with AL amyloidosis. Bone Marrow Transplant, 2000, 25(12):1299–1301.
[115] Windrum P, Morris T. Severe neurotoxicity because of dimethyl sulphoxide following peripheral blood stem cell transplantation. Bone Marrow Transplant, 2003, 31(4):315.
[116] Rodbell M. Metabolism of isolated fat cells. II. The similar effects of phospholipase C (*Clostridium perfringens* alpha toxin) and of insulin on glucose and amino acid metabolism. J Biol Chem, 1966, 241(1):130–139.
[117] Van RL, Roncari DA. Isolation of fat cell precursors from adult rat adipose tissue. Cell Tissue Res, 1977, 181(2):197–203.
[118] Deslex S, Negrel R, Vannier C, Etienne J, Ailhaud G. Differentiation of human adipocyte precursors in a chemically defined serum-free medium. Int J Obes, 1987, 11(1):19–27.
[119] Hauner H, Entenmann G, Wabitsch M, Gaillard D, Ailhaud G, Negrel R, Pfeiffer EF. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J Clin Invest, 1989, 84(5):1663–1670.
[120] Williams SK, Wang TF, Castrillo R, Jarrell BE. Liposuction-derived human fat used for vascular graft sodding contains endothelial cells and not mesothelial cells as the major cell type. J Vasc Surg, 1994, 19(5):916–923.

[121] Moore JH Jr, Kolaczynski JW, Morales LM, Considine RV, Pietrzkowski Z, Noto PF, Caro JF. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. Aesthetic Plast Surg, 1995, 19(4):335–339.
[122] Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clin Plast Surg, 1999, 26(4): 587–603, *viii*.
[123] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science, 1999, 284(5411):143–147.
[124] Sen A, Lea-Currie YR, Sujkowska D, Franklin DM, Wilkison WO, Halvorsen YD, Gimble JM. Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous. J Cell Biochem, 2001, 81(2):312–329.
[125] Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng, 2001, 7(2):211–228.
[126] Halvorsen YD, Franklin D, Bond AL, Hitt DC, Auchter C, Boskey AL, Paschalis EP, Wilkison WO, Gimble JM. Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived stromal cells. Tissue Eng, 2001, 7(6):729–741.
[127] Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Characterization of surface protein expression on human adipose tissue-derived stromal cells. J Cell Physiol, 2001, 189(1):54–63.
[128] Gimble JM, Guilak F. Differentiation potential of adipose derived adult stem (ADAS) cells. Curr Top Dev Biol, 2003, 58:137–160.
[129] Miranville A, Heeschen C, Sengenès C, Curat CA, Busse R, Bouloumié A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation, 2004, 110(3):349–355.
[130] Kakagia D, Pallua N. Autologous fat grafting: in search of the optimal technique. Surg Innov, 2014, 21(3):327–336.
[131] Pu LL, Coleman SR, Cui X, Ferguson RE Jr, Vasconez HC. Autologous fat grafts harvested and refined by the Coleman technique: a comparative study. Plast Reconstr Surg, 2008, 122(3):932–937.
[132] Ozsoy Z, Kul Z, Bilir A. The role of cannula diameter in improved adipocyte viability: a quantitative analysis. Aesthet Surg J, 2006, 26(3):287–289.
[133] Campbell GLM, Laudenslager N, Newman J. The effect of mechanical stress on adipocyte morphology and metabolism. Am J Cosmet Surg, 1987, 4(2):89–94.
[134] Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study. J Plast Reconstr Aesthet Surg, 2009, 62(9):1210–1214.
[135] Dasiou-Plakida D. Fat injections for facial rejuvenation: 17 years experience in 1720 patients. J Cosmet Dermatol, 2003, 2(3–4):119–125.
[136] Coleman SR, Mazzola RF (eds). Fat injection: from filling to regeneration. Quality Medical Publishing, St. Louis, MO, 2009, 373–422.
[137] Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, Declercq H. Nanofat grafting: basic research and clinical applications. Plast Reconstr Surg, 2013, 132(4):1017–1026.
[138] Gause TM 2nd, Kling RE, Sivak WN, Marra KG, Rubin JP, Kokai LE. Particle size in fat graft retention: a review on the impact of harvesting technique in lipofilling surgical outcomes. Adipocyte, 2014, 3(4):273–279.
[139] Crawford JL, Hubbard BA, Colbert SH, Puckett CL. Fine tuning lipoaspirate viability for fat grafting. Plast Reconstr Surg, 2010, 126(4):1342–1348.
[140] Hamza A, Lohsiriwat V, Rietjens M. Lipofilling in breast cancer surgery. Gland Surg, 2013, 2(1):7–14.
[141] Agostini T, Lazzeri D, Pini A, Marino G, Li Quattrini A, Bani D, Dini M. Wet and dry techniques for structural fat graft harvesting: histomorphometric and cell viability assessments of lipoaspirated samples. Plast Reconstr Surg, 2012, 130(2): 331e–339e.
[142] Klein JA. Tumescent technique for local anesthesia improves safety in large-volume liposuction. Plast Reconstr Surg, 1993, 92(6):1085–1098; discussion 1099–1100.
[143] Mojallal A, Foyatier JL. [The effect of different factors on the survival of transplanted adipocytes]. Ann Chir Plast Esthet, 2004, 49(5):426–436.
[144] Banyard DA, Salibian AA, Widgerow AD, Evans GRD. Implications for human adipose-derived stem cells in plastic surgery. J Cell Mol Med, 2015, 19(1):21–30.
[145] Raposio E, Bonomini S, Calderazzi F. Isolation of autologous adipose tissue-derived mesenchymal stem cells for bone repair. Orthop Traumatol Surg Res, 2016, 102(7):909–912.
[146] Chang KA, Kim HJ, Joo Y, Ha S, Suh YH. The therapeutic effects of human adipose-derived stem cells in Alzheimer's disease mouse models. Neurodegener Dis, 2014, 13(2–3): 99–102.
[147] Rada T, Reis RL, Gomes ME. Novel method for the isolation of adipose stem cells (ASCs). J Tissue Eng Regen Med, 2009, 3(2):158–159.
[148] Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg, 2010, 63(8):1375–1381.
[149] Cleveland EC, Albano NJ, Hazen A. Roll, spin, wash, or filter? Processing of lipoaspirate for autologous fat grafting: an updated, evidence-based review of the literature. Plast Reconstr Surg, 2015, 136(4):706–713.
[150] Gerth DJ, King B, Rabach L, Glasgold RA, Glasgold MJ. Long-term volumetric retention of autologous fat grafting processed with closed-membrane filtration. Aesthet Surg J, 2014, 34(7):985–994.
[151] Ferraro GA, de Francesco F, Tirino V Cataldo C, Rossano F, Nicoletti G, D'Andrea F. Effects of a new centrifugation method on adipose cell viability for autologous fat grafting. Aesthetic Plast Surg, 2011, 35(3):341–348.
[152] Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The current state of fat grafting: a review of harvesting, processing, and injection techniques. Plast Reconstr Surg, 2015, 136(4):897–912.
[153] Hoareau L, Bencharif K, Girard AK, Gence L, Delarue P, Hulard O, Festy F, Roche R. Effect of centrifugation and washing on adipose graft viability: a new method to improve graft efficiency. J Plast Reconstr Aesthet Surg, 2013, 66(5): 712–719.
[154] Pham PV. Adipose stem cells in the clinic. Biomed Res Ther, 2014, 1(2):57–70.
[155] Thirumala S, Goebel WS, Woods EJ. Clinical grade adult stem cell banking. Organogenesis, 2009, 5(3):143–154.
[156] ***. ClinicalTrials.gov. U.S. National Library of Medicine, available at: https://clinicaltrials.gov.
[157] Lendeckel S, Jödicke A, Christophis P, Heidinger K, Wolff J, Fraser JK, Hedrick MH, Berthold L, Howaldt HP. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report. J Craniomaxillofac Surg, 2004, 32(6):370–373.
[158] Thesleff T, Lehtimäki K, Niskakangas T, Mannerström B, Miettinen S, Suuronen R, Öhman J. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. Neurosurgery, 2011, 68(6):1535–1540.
[159] Mesimäki K, Lindroos B, Törnwall J, Mauno J, Lindqvist C, Kontio R, Miettinen S, Suuronen R. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int J Oral Maxillofac Surg, 2009, 38(3):201–209.
[160] Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. Stem Cell Res Ther, 2010, 1(2):19.
[161] Pak J. Autologous adipose tissue-derived stem cells induce persistent bone-like tissue in osteonecrotic femoral heads. Pain Physician, 2012, 15(1):75–85.
[162] Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: a case series. J Med Case Rep, 2011, 5:296.
[163] Valina C, Pinkernell K, Song YH, Bai X, Sadat S, Campeau RJ, Le Jemtel TH, Alt E. Intracoronary administration of autologous adipose tissue-derived stem cells improves left ventricular function, perfusion, and remodelling after acute myocardial infarction. Eur Heart J, 2007, 28(21):2667–2677.
[164] Alt E, Pinkernell K, Scharlau M, Coleman M, Fotuhi P, Nabzdyk C, Matthias N, Gehmert S, Song YH. Effect of

freshly isolated autologous tissue resident stromal cells on cardiac function and perfusion following acute myocardial infarction. Int J Cardiol, 2010, 144(1):26–35.
[165] Ii M, Horii M, Yokoyama A, Shoji T, Mifune Y, Kawamoto A, Michio A, Asahara T. Synergistic effect of adipose-derived stem cell therapy and bone marrow progenitor recruitment in ischemic heart. Lab Invest, 2011, 91(4):539–552.
[166] Schenke-Layland K, Strem BM, Jordan MC, Deemedio MT, Hedrick MH, Roos KP, Fraser JK, Maclellan WR. Adipose tissue-derived cells improve cardiac function following myocardial infarction. J Surg Res, 2009, 153(2):217–223.
[167] Sridhar P, Hedrick M, Baker T, Perin J, Conlan B. Adipose-derived regenerative cells for the treatment of patients with non-revascularisable ischaemic cardiomyopathy – the PRECISE Trial. Interv Cardiol Rev, 2012, 7(2):77–80.
[168] Moon MH, Kim SY, Kim YJ, Kim SJ, Lee JB, Bae YC, Sung SM, Jung JS. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. Cell Physiol Biochem, 2006, 17(5–6):279–290.
[169] Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, Pell CL, Johnstone BH, Considine RV, March KL. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation, 2004, 109(10): 1292–1298.
[170] Traktuev DO, Prater DN, Merfeld-Clauss S, Sanjeevaiah AR, Saadatzadeh MR, Murphy M, Johnstone BH, Ingram DA, March KL. Robust functional vascular network formation *in vivo* by cooperation of adipose progenitor and endothelial cells. Circ Res, 2009, 104(12):1410–1420.
[171] Planat-Benard V, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Pénicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation, 2004, 109(5):656–663.
[172] Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, Johnstone BH, March KL. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res, 2008, 102(1):77–85.
[173] Tabit CJ, Slack GC, Fan K, Wan DC, Bradley JP. Fat grafting *versus* adipose-derived stem cell therapy: distinguishing indications, techniques, and outcomes. Aesthetic Plast Surg, 2012, 36(3):704–713.
[174] Tiryaki T, Findikli N, Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: a preliminary report. Aesthetetic Plast Surg, 2011, 35(6):965–971.
[175] Koh KS, Oh TS, Kim H, Chung IW, Lee KW, Lee HB, Park EJ, Jung JS, Shin IS, Ra JC, Choi JW. Clinical application of human adipose tissue-derived mesenchymal stem cells in progressive hemifacial atrophy (Parry–Romberg disease) with microfat grafting techniques using 3-dimensional computed tomography and 3-dimensional camera. Ann Plast Surg, 2012, 69(3):331–337.
[176] Kim M, Kim I, Lee SK, Bang SI, Lim SY. Clinical trial of autologous differentiated adipocytes from stem cells derived from human adipose tissue. Dermatol Surg, 2011, 37(6): 750–759.
[177] Altman AM, Yan Y, Matthias N, Bai X, Rios C, Mathur AB, Song YH, Alt EU. IFATS collection: human adipose-derived stem cells seeded on a silk fibroin–chitosan scaffold enhance wound repair in a murine soft tissue injury model. Stem Cells, 2009, 27(1):250–258.
[178] Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, Park JS. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci, 2007, 48(1):15–24.
[179] Maharlooei MK, Bagheri M, Solhjou Z, Jahromi BM, Akrami M, Rohani L, Monabati A, Noorafshan A, Omrani GR. Adipose tissue derived mesenchymal stem cell (AD-MSC) promotes skin wound healing in diabetic rats. Diab Res Clin Pract, 2011, 93(2):228–234.
[180] Nie C, Yang D, Xu J, Si Z, Jin X, Zhang J. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. Cell Transplant, 2011, 20(2):205–216.
[181] Jackson WM, Nesti LJ, Tuan RS. Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. Stem Cells Transl Med, 2012, 1(1):44–50.
[182] Kim WS, Park BS, Sung JH. The wound-healing and antioxidant effects of adipose-derived stem cells. Exp Opin Biol Ther, 2009, 9(7):879–887.
[183] Akita S, Akino K, Hirano A, Ohtsuru A, Yamashita S. Non-cultured autologous adipose-derived stem cells therapy for chronic radiation injury. Stem Cells Int, 2010, 2010:532704.
[184] Akita S, Yoshimoto H, Ohtsuru A, Hirano A, Yamashita S. Autologous adipose-derived regenerative cells are effective for chronic intractable radiation injuries. Radiat Prot Dosimetry, 2012, 151(4):656–660.
[185] Cervelli V, De Angelis B, Lucarini L, Spallone D, Balzani A, Palla L, Gentile P, Cerulli P. Tissue regeneration in loss of substance on the lower limbs through use of platelet-rich plasma, stem cells from adipose tissue, and hyaluronic acid. Adv Skin Wound Care, 2010, 23(6):262–272.
[186] Rigotti G, Marchi A, Galiè M, Baroni G, Benati D, Krampera M, Pasini A, Sbarbati A. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg, 2007, 119(5):1409–1422; discussion 1423–1424.
[187] Lin CS, Lin G, Lue TF. Allogeneic and xenogeneic transplantation of adipose-derived stem cells in immunocompetent recipients without immunosuppressants. Stem Cells Dev, 2012, 21(15):2770–2778.
[188] Griffin MD, Ritter T, Mahon BP. Immunological aspects of allogeneic mesenchymal stem cell therapies. Hum Gene Ther, 2010, 21(12):1641–1655.
[189] Fang B, Song Y, Lin Q, Zhang Y, Cao Y, Zhao RC, Ma Y. Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-*vs.*-host disease in two children. Pediatr Transplant, 2007, 11(7):814–817.
[190] Tholpady SS, Ogle RC, Katz AJ. Adipose stem cells and solid organ transplantation. Curr Opin Organ Transplant, 2009, 14(1):51–55.
[191] Fang B, Song YP, Liao LM, Han Q, Zhao RC. Treatment of severe therapy-resistant acute graft-*versus*-host disease with human adipose tissue-derived mesenchymal stem cells. Bone Marrow Transplant, 2006, 38(5):389–390.
[192] Fang B, Song Y, Liao L, Zhang Y, Zhao RC. Favorable response to human adipose tissue-derived mesenchymal stem cells in steroid-refractory acute graft-*versus*-host disease. Transplant Proc, 2007, 39(10):3358–3362.
[193] Fang B, Song Y, Zhao RC, Han Q, Lin Q. Using human adipose tissue-derived mesenchymal stem cells as salvage therapy for hepatic graft-*versus*-host disease resembling acute hepatitis. Transplant Proc, 2007, 39(5):1710–1713.
[194] Fang B, Song Y, Zhao RC, Han Q, Cao Y. Treatment of resistant pure red cell aplasia after major ABO-incompatible bone marrow transplantation with human adipose tissue-derived mesenchymal stem cells. Am J Hematol, 2007, 82(8):772–773.
[195] Fang B, Song YP, Li N, Li J, Han Q, Zhao RC. Resolution of refractory chronic autoimmune thrombocytopenic purpura following mesenchymal stem cell transplantation: a case report. Transplant Proc, 2009, 41(5):1827–1830.
[196] Garcia-Olmo D, Garcia-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. Expert Opin Biol Ther, 2008, 8(9):1417–1423.
[197] Garcia-Olmo D, Herreros D, Pascual M, Pascual I, De-La-Quintana P, Trebol J, Garcia-Arranz M. Treatment of enterocutaneous fistula in Crohn's disease with adipose-derived stem cells: a comparison of protocols with and without cell expansion. Int J Colorectal Dis, 2009, 24(1):27–30.
[198] McIntosh K, Zvonic S, Garrett S, Mitchell JB, Floyd ZE, Hammill L, Kloster A, Di Halvorsen Y, Ting JP, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM. The immunogenicity of human adipose-derived cells: temporal changes *in vitro*. Stem Cells, 2006, 24(5):1246–1253.
[199] Vanikar AV, Dave SD, Thakkar UG, Trivedi HL. Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus. Stem Cells Int, 2010, 2010:582382.

[200] Han C, Zhang L, Song L, Liu Y, Zou W, Piao H, Liu J. Human adipose-derived mesenchymal stem cells: a better cell source for nervous system regeneration. Chin Med J (Engl), 2014, 127(2):329–337.
[201] Kang SK, Shin MJ, Jung JS, Kim YG, Kim CH. Autologous adipose tissue-derived stromal cells for treatment of spinal cord injury. Stem Cells Dev, 2006, 15(4):583–594.
[202] Moscatello DK, Schiavi J, Marquart JD, Lawrence N. Collagenase-assisted fat dissociation for autologous fat transfer. Dermatol Surg, 2008, 34(10):314–321; discussion 1321–1322.
[203] Youn S, Shin JI, Kim JD, Kim JT, Kim YH. Correction of infraorbital dark circles using collagenase-digested fat cell grafts. Dermatol Surg, 2013, 39(5):766–772.
[204] Boureaux E, Chaput B, Bannani S, Herlin C, De Runz A, Carloni R, Mortemousque B, Mouriaux F, Watier E, Bertheuil N. Eyelid fat grafting: indications, operative technique and complications; a systematic review. J Craniomaxillofac Surg, 2016, 44(4):374–380.
[205] Hoang D, Orgel MI, Kulber DA. Hand rejuvenation: a comprehensive review of fat grafting. J Hand Surg Am, 2016, 41(5):639–644.
[206] Kao WP, Lin YN, Lin TY, Huang YH, Chou CK, Takahashi H, Shieh TY, Chang KP, Lee SS, Lai CS, Lin SD, Lin TM. Microautologous fat transplantation for primary augmentation rhinoplasty: long-term monitoring of 198 Asian patients. Aesthet Surg J, 2016, 36(6):648–656.
[207] Tzouvelekis A, Paspaliaris V, Koliakos G, Ntolios P, Bouros E, Oikonomou A, Zissimopoulos A, Boussios N, Dardzinski B, Gritzalis D, Antoniadis A, Froudarakis M, Kolios G, Bouros D. A prospective, non-randomized, no placebo-controlled, phase Ib clinical trial to study the safety of the adipose derived stromal cells–stromal vascular fraction in idiopathic pulmonary fibrosis. J Transl Med, 2013, 11:171.
[208] Rodriguez AM, Pisani D, Dechesne CA, Turc-Carel C, Kurzenne JY, Wdziekonski B, Villageois A, Bagnis C, Breittmayer JP, Groux H, Ailhaud G, Dani C. Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. J Exp Med, 2005, 201(9):1397–1405.
[209] Mizuno H. The potential for treatment of skeletal muscle disorders with adipose-derived stem cells. Curr Stem Cell Res Ther, 2010, 5(2):133–136.
[210] Vieira NM, Brandalise V, Zucconi E, Jazedje T, Secco M, Nunes VA, Strauss BE, Vainzof M, Zatz M. Human multipotent adipose-derived stem cells restore dystrophin expression of Duchenne skeletal-muscle cells *in vitro*. Biol Cell, 2008, 100(4):231–241.
[211] Casiraghi F, Remuzzi G, Abbate M, Perico N. Multipotent mesenchymal stromal cell therapy and risk of malignancies. Stem Cell Rev, 2013, 9(1):65–79.
[212] Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F 3rd. Concise review: dissecting a discrepancy in the literature: do mesenchymal stem cells support or suppress tumor growth? Stem Cells, 2011, 29(1):11–19.
[213] Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, Phinney DG, Simmons PJ, Sensebe L, Keating A. Defining the risks of mesenchymal stromal cell therapy. Cytotherapy, 2010, 12(5):576–578.
[214] Rubio D, Garcia-Castro J, Martín MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. Cancer Res, 2005, 65(8): 3035–3039.
[215] Garcia S, Bernad A, Martín MC, Cigudosa JC, Garcia-Castro J, de la Fuente R. Pitfalls in spontaneous *in vitro* transformation of human mesenchymal stem cells. Exp Cell Res, 2010, 316(9):1648–1650.
[216] U.S. Food and Drug Administration. Draft guidance for industry: Current Good Tissue Practice (CGTP) and additional requirements for manufacturers of Human Cells, Tissues, and cellular and tissue-based Products (HCT/Ps). Available at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073366.htm.
[217] European Medicines Agency (EMA). Patient health protection. EMA/129056/2013, 4 April 2013.
[218] Committee for Advanced Therapies (CAT). Overview of comments received on “Reflection paper on classification of advanced therapy medicinal products”. EMA/CAT/600280/2010 rev. 1, 2015.

Corresponding author
Eleonora Dronca, MD, PhD, Department of Molecular Sciences, “Iuliu Haţieganu” University of Medicine and Pharmacy, 6 Louis Pasteur Street, 400349 Cluj-Napoca, Romania; Phone +40374–834 114, Fax +40264–597 257, e-mail: eleonora.dronca@umfcluj.ro

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