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Adipose Derived Stem Cells: Current State of the Art and Prospective Role in Regenerative Medicine and Tissue Engineering

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1. Introduction

1.1. Adipose tissue: The Good, the Bad, the Ugly

Excessive body fat has been socially recognized for ages as a symbol of wealth and prosperity. Clues of these concepts may be found in arts and literature. In addition, it has been substantially ignored by scientists, anatomists and physicians for many centuries. As a matter of fact only a minimal number of medical reports focused on "fat" have been historically handed down. Nowadays, however, adipose tissue has become a growing point of most interest for research-ers and physicians worldwide. Notably, societies and health care systems are facing a severe pandemic rise of obesity and of several associated co-morbidities such as cardiovascular disease, diabetes, metabolic disorders and cancer. Fat and misregulation of adipose-related pathways are recognized as key elements in each of these processes. Importantly, the role of adipose tissue has progressively evolved from being a passive energy store to representing an important endocrine organ that directly modulates metabolism and immunity towards an healthy phenotype or leading to pathologic processes. The investigation of the physiologic-pathologic attitudes of adipose tissue is currently among most relevant scientific targets of researchers, endocrinologists and bariatric surgeons. Beside, in the last fifteen years adipose tissue has been reappraised also for a different reason. In fact, nearly forty years after the identification of bone marrow stem cells, it has been gathering attention for the opportunity to obtain autologous pluripotent adipose-derived stromal stem cells (ADSCs). This population of cells has been extensively investigated and it currently holds out many hopes for prospective

stem cell therapies for the repair and regeneration of various tissues and organs in a large number of different diseases. Thus, over the past years, this field has become a very active and attractive area of clinical and experimental research, providing significant outcomes and reaching important milestones. Today adipose tissue embodies an hot spot of regenerative medicine that may give rise to a new era of active stem cell therapy.

2. Purpose

2.1. Meeting the adipose tissue

Giving the increasing amount of experimental and clinical data regarding adipose tissue and ADSCs, in this chapter we are going to briefly review the concepts and the insights behind the role of adipose tissue in regenerative medicine and tissue engineering. In particular we are going to focus the attention on current cutting edge translational research from bench to bedside, including the investigation of biological properties of ADSCs, the state of art of their manipulation, the latest progresses in their clinical adoption, the development of bio-engineered products and the actual therapeutic prospective opportunities.

3. Basic science background

3.1. The outline and the anatomy of adipose tissue

Adipose tissue is a complex and multi-depot organ, constituted for one third by mature adipocytes and for the other two thirds by a combination of a large variety of other cells. [1] Among represented cell lines are included small blood vessels, nervous cells, fibroblasts and, importantly, adipocyte progenitor cells, also known as preadipocytes or Adipose Derived Stem Cells (ADSCs). Evolution has preserved in mammals two histologically different qualities of adipose tissue: white adipose tissue (WAT) and brown adipose tissue (BAT), which are composed by different types of mature adipocytes [Table 1]. In particular, white adipocytes are spherical, having a diameter ranging between 30 and 70 µm according to the amount of lipid depots, and lipids within the cells are organized in a single large "uni-locular" droplet, the size of which can exceed 50 µm. Thus, the lipid droplet occupies the vast majority of the whole intracellular space, pushing the remaining cytoplasm and nucleus into a thin marginal rim. On the other hand, brown adipocytes are polygonal with a centrally placed nucleus and their cellular size ranges from 20 to 40 µm. They accumulate lipids in smaller "multi-locular" droplets and they are rich of specific mitochondria, containing the protein UCP-1 which is responsible for uncoupling of oxidative phosporylation and production of heat. WAT and BAT are both innervated by noradrenergic fibers of the sympathetic nervous system. As for the vascularization of adipose tissue, white adipocytes are organized in collections of fat lobules, each supplied by a selective arteriole and surrounded by septae of connective tissue. An individual adipocyte is supplied by an adjacent capillary and it is associated to a glycoprotein layer, reticular fibrils, fibroblasts, mastocytes and macrophages. Compared to WAT, BAT provides a more extensive vascular tree, characterized by dense multiple capillaries. The relevant vascularization of the latter in combination with the

presence of a significantly high number of mitochondria, account for the typical "brown" color. WAT and BAT have also different roles in energy metabolism. Primary function of white adipocytes is to store excess energy as lipid, which is then mobilized in response to metabolic needs. Brown adipocytes, on the other hand, use accumulated lipids primarily as a source of energy released in the form of heat. WAT can be found in several anatomically distinct and separate collections, or "depots." There are two major anatomic subdivisions of these depots, each showing unique anatomic, metabolic, endocrine, paracrine, and autocrine properties: intra-abdominal or visceral adipose tissue and subcutaneous adipose tissue. In addition, WAT can also be found in small amounts of fatty layers surrounding other organs, such as the heart, kidney and genitalia. Intra-peritoneal fat, composed of omental and mesenteric adipose tissue, comprises the vast majority of visceral fat. Importantly, subcutaneous adipose tissue shows different structural features in different anatomical districts. [2] In fact, fat depots in the abdominal area are characterized by the presence of large adipocytes, densely packed together and surrounded by a poor stromal (collagen) network. Instead, in more localized depots (such as throcanteric areas, the sovra-pubic area, arm pits, medial regions of the knees, tights, arms, pectoral and mammary areas) adipocytes present a smaller diameter, a more represented stromal component and a more extensive vascular network. BAT in newborns and children can be found in several body areas. However, while in other small mammals these depots persist during growth, in humans brown adipocytes undergo a morphologic transformation, rapidly accumulating lipids, becoming uni-locular and losing their typical ultrastructural and molecular properties, including mitochondria [Figure 1.]. As a consequence, there are no discrete collections of BAT that can be found in human adults.

	White Adipocyte	Brown Adipocyte
Shape	Spherical	Polygonal
Diameter	30-70 µm	20-40 µm
Ultra-structure	One large "unilocular" lipid droplet, cytoplasm and nucleus compressed into a thin visible rim	Multiple smaller "multilocular" droplets, high content of mitochondria, centrally placed nucleus
Innervation	Noradrenergic fibers, confined to capillary wall	Noradrenergic fibers, directly interfacing plasma membrane
Vascularization	Supplied by an adjacent capillary	Richer vascular tree, dense with multiple capillaries
Main function	Store excess energy as lipids	Thermogenesis
Localization	Visceral compartment (intraperitoneal, retroperitoneal, around organs) and subcutaneous compartment	Several areas in newborn, no discrete collections in adult. Probably isolated cells scattered between WAT depots

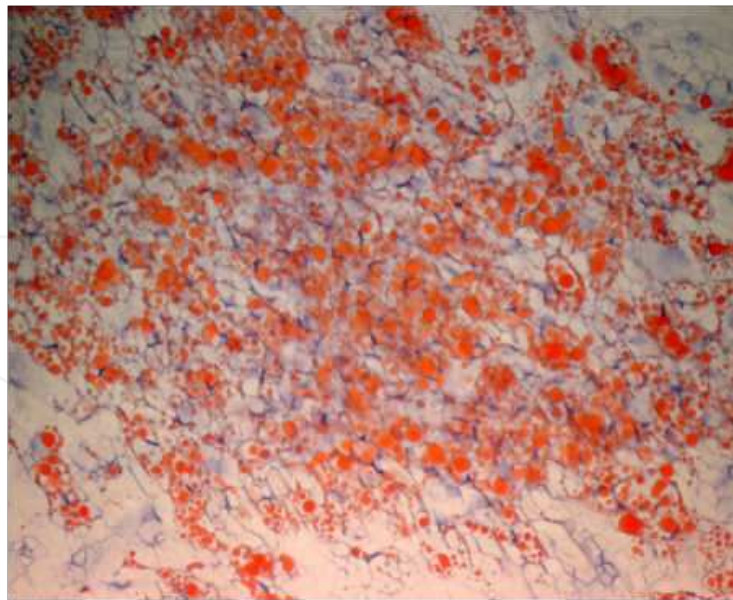**Table 1.** Main differences between White and Brown adipocytes.

Figure 1. Monolayered culture of adipocytes in vitro with adipogenic medium.

3.2. The living image of adipose derived stem cells

The understanding of biochemical characteristics, molecular/cellular biology, immune-biological characteristics and phenotype of adipose tissue has significantly advanced in the last years. Adipose tissue has shown to consist mostly of cells of mesenchymal origin with few others endothelial cells, smooth muscle cells and pericytes, all showing low levels of cell senescence. Adipose tissue derives from the mesodermal layer of the embryo and develops both during pre-natal and post-natal growth. The microscopic location of the adipogenic progenitor cells in the adult is still controversial. [3] It remains to be proven whether the origin of the cells correlates with endothelial, pericytic or stromal compartments. A large number of surface antigens are in common with endothelial cells, suggesting a common origin. According to some researchers, adipogenic progenitor cells could be released directly by the bone marrow and distributed systemically by blood flow: experimental evidences of bone marrow derived-cells capable of differentiating into adipocytes in vivo have already been described but the contribution of these circulating cells to the overall growth and development of adipose tissue is still under investigation. Mesenchymal stem cells (MSC) were first described as immature cells in the bone marrow, capable to give rise to mesenchymal lineages such as osteoblasts, chondrocytes and adipocytes. [4] MSCs represent a small fraction of nucleated cells of human bone marrow (0.01%-0,0001%). MSCs are defined by three minimal criteria, as established by the International Society for Cellular Therapy in 2005: adherence to plastic dishes, specific surface antigen (CD73+, CD90+, CD105+, CD45-, CD34-, CD14 or CD11b-, CD79- or CD19-, HLA-DR) and in vitro capability to give rise to adipocytes, osteoblasts and chondrocytes. A similar protocol has been used for a long time to isolate adipose tissue progenitors: the resulting immature adherent cells were thus called pre-adipocytes. To obtain these cells fat pads are minced and digested with collagenase, separating an upper layer of floating mature adipocytes

from a lower layer of pelleted stromal vascular fraction (SVF). [5] The SVF is an heterogeneous cell population of circulating blood cells, fibroblasts, pericytes, endothelial cells and pre-adipocytes. Pre-adipocytes may be isolated from the SVF by plating and washing. This cell population, adopting appropriate differentiating agents, can give rise to mature adipocytes, demonstrating their nature of adipose progenitors. Cell cultures have provided evidence of regenerative capacities in both the heterogeneous stromal vascular fraction (SVF) and in the more homogeneous adipose-derived stem cells (ADSCs). In 2002 pre-adipocytes were better characterized and they were demonstrated to show clear multi-potency potential: thus, they were named Adipose Derived Stem Cells (ADSCs). [6] In particular, ADSCs represent a mesodermal stem cell population with clonal mesodermal, ectodermal, and endodermal potentials capabilities that express multiple CD marker antigens similar to those of other mesenchymal stem cells as those residing in bone marrow. Several investigations have reported a differentiation into adipogenic, osteogenic, chondrogenic and myogenic lineages in vitro by means of specific culture media. In particular, the potential to differentiate into non-mesodermal lineages is exciting. The differentiation into neural precursors, which are of an ectodermal origin, has been described. In addition, evidence of differentiation into hepato-cytes, pancreatic islet cells, endothelial cells and other epithelial cells has been provided in different reports. By definition, a stem cell is characterized by the ability to self-renew and to differentiate along multiple lineage pathways. Since the self-renewal of ADSCs has not been fully established yet, it is accepted that some investigators may use the same acronym to mean "adipose-derived stromal cells", in agreement with the statement of the International Society for Cellular Therapy. Indeed, ADSCs present several differences from MSCs at genomic, proteomic and functional levels. For instance, during the earliest rounds of proliferation, ADSCs express the CD34 antigen: the frequency of these cells is much higher (100 to 500 folds higher) than that of MSCs in the bone marrow. In addition, MSCs are probably more committed towards osteoblastic and chondrogenic lineages than ADSCs. Thus, although numerous author use the same term "MSCs" both for cells derived from bone marrow and for those derived from adipose tissue, MSCs and ADSCs are probably two distinct cell populations. A more precise definition of ADSCs, based on their immune-phenotype and/or differentiation capabilities, has not been yet provided. Some authors believe that ADSCs are a heterogeneous group of progenitor cells with differences in their stem cell potential. Thus, ADSCs and SVFs cells represent an autologous alternative to pluri-potent embryonic stem cells with a multi-lineage differentiation potential, a significant therapeutic impact and a critical role in the rapidly expanding fields of tissue engineering and regenerative medicine. Significantly, further investigations are needed to better clarify these aspects. Importantly, the most important characteristics of ADSCs, with a possible interest for clinical applications, comprise their multi-potency, secretory functions and immune-modulatory capabilities.

3.2.1. Differentiation potential of ADSCs

ADSCs, like MSCs, have the ability to differentiate into mesodermal cells, such as adipocytes, fibroblasts, myocytes, osteocytes and chondrocytes, in a process called lineage-specific differentiation. The increasing evidence for the ability of ADSCs to differentiate into cells of non-mesodermal origin such as neurons, endocrine pancreatic cells, hepatocytes, endothelial

cells and cardiac myocytes, is surprising. This process is called "cross-differentiation". Lineage-specific differentiation can be tracked at a molecular level by the expression of key transcription factors of mature tissues. The earlier stages of differentiation, named "allocation" or "commitment", that drive the ADSCs into the specialized lineage are not completely known yet. In vitro, the differentiation of multi-potent cells into a desirable cell phenotype can be obtained by appropriate culture conditions and stimulation with a cocktail of known differentiating agents [Table 2].

Type of differentiation	Stimulating factors
Adipogenic	Insulin; isobutylmethylxanthine (IBMX) ; dexamethasone; rosiglitazone; indomethacin.
Osteogenic	Dexamethasone; β-glycerophosphate; vitamin D3; bone morphogenetic protein (BMP-2)
Chondrogenic	insulin growth factor (IGF); BMPs; transforming growth factor-β (TGF-β)
Myogenic/cardiomyogenic	Dexamethasone; hydrocortisone; IL-3; IL-6
Vascular/endothelial	Specific environment
Neurogenic	Valproic acid; epidermal growth factor (EGF); fibroblast growth factor (FGF); nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF)
Tendinous	FGF; platelet derived growth factor (PDGF-BB); EGF; TGF-β; IGF-1; BMPs

Table 2. Experimental growth factors used for differentiation of ADSCs in different cell lineages.

- Adipogenic differentiation

ADSCs have an exceptional potential for differentiation into mature adipocytes, which is very promising in developing techniques for repairing soft-tissue defects. [7] Differentiation can be induced by a large variety of substances, including insulin, dexamethasone, rosiglitazone and indomethacin. During differentiation ADSCs, initially showing a fibroblast-like spindle or stellate shape, undergo morphologic changes with the appearance of one or more lipid vacuoles and they begin to express several genes and proteins characterizing the mature adipocyte, including leptin, peroxisome-proliferating activated receptor γ (PPARγ), glucose transporter type 4 (GLUT4) and glycerol-3-phosphate dehydrogenase (GPDH).

- Osteogenic differentiation

Osteogenic differentiation can be induced in vitro by supplementing the culture medium with dexamethasone, β-glycerophosphate and vitamin D3. The acquisition of the osteoblast phenotype is accompanied by expression of specific genes and proteins, including alkaline phosphatase, type I collagen, osteopontin, osteonectin, and Runx2. Osteogenic differentiation may also be obtained by transfection of osteogenic lineage-determining genes (BMP2 and Runx2): this approach has proved to be effective both in vitro and in vivo in a large number

of reports. These experimental findings hold great promise for the use of ADSCs in bone regeneration.

- Chondrogenic differentiation

Insulin growth factor (IGF), bone morphogenetic proteins (BMPs), and transforming growth factor-β (TGF-β) have shown to induce chondrogenic differentiation of ADSCs when added to the culture medium. Chondrogenic differentiation occurs also by seeding ADSCs into poly-glycolic acid (PGA) scaffolds, as it was largely demonstrated in several other in vitro models and in vivo in nude mice.

- Differentiation into other lineages

Terminally differentiated myoblasts can be obtained in vitro, showing the ability to form multinucleated myotubules and to shrink/diastole under the influence of atropine. This property of ADSCs is of particular interest for the treatment of genetic muscular dystrophies: preclinical in vivo studies on animal models are currently ongoing. In addition, other studies have focused on the capability of ADSCs to differentiate into cardiomyocytes with a possible application in heart regeneration or repair after an ischemic injury. Furthermore, endothelial regeneration is another important field of research: ADSCs have shown to be able to differentiate into endothelial cells and to secrete several pro-angiogenic factors, like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Differentiation into neuron-like cells has also been reported by different authors: ADSCs may acquire a neural-like morphology and they may express several proteins specific for the neuronal phenotype (Neuron Specific Enolase; Neuron Specific Nuclear Protein). Finally, some studies have explored the chance for ADSCs to differentiate into pancreatic islet cells, hepatocytes and epithelial cells with the purpose to find an alternative cellular therapy for diseases such as diabetes mellitus and liver disfunction: data and outcomes are however still preliminary and lacking of strong evidence.

3.2.2. ADSCs as a secretome

Importance of ADSCs does not only reside in their potential to differentiate in mature lineages. Similarly to the original adipose tissue from which they can be isolated, ADSCs have shown to act as a "secretome", accurately regulating proteins and growth factors secreted into the extracellular milieu and having a relevant impact on different organs and systems within the human body [Table 3.]. [8] Trophic effects of ADSCs include stimulation of angiogenesis, hematopoietic support, gene transfer and suppression of inflammation. Indeed ADSCs represent a source of several cytokine/soluble factors regulating the survival and differentiation of various endogenous cells/tissues. A large number of these molecules have been related to the regenerative attitude of ADSCs: among these, we may include hepatocyte growth factor (HGF), granulocyte and macrophage colony stimulating factors, interleukins (ILs) 6, 7, 8 and 11, tumor necrosis factor-alpha (TNF-alpha), vascular endothelial growth factor (VEGF), brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), adipokines and others. Full characterization of the secretory profile of ADSCs, either by immune-enzymatic techniques (ELISA) or by mass spectrometry, is still object of investigation. Several adipokines such as

adiponectin, angiotensin, cathepsin D, penetraxin, pregnancy zone protein and retinol binding protein, as well as stromal cell-derived growth factor (CXCL12) have been found in the conditioned media of ADSCs differentiating towards the adipocyte lineage. ADSCs secrete also oher different well characterized cytokines (GM-CSF, TGF-β, PGE2, IGF-1) and their release can be modulated by exposure to different agents, such as b-FGF and EGF or inflammatory stimula, like lipopolysaccharide (LPS). The role of these and other factors has been investigated by multiple studies regarding one or more possible applications of ADSCs in the field of regenerative medicine. Brain Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), Glial Derived Neurotrophic Factor (GDNF) are thought to be important molecules secreted by ADSCs mediating neurotrophic effects and modulating in animal models of Parkinson Disease the recovery after hypoxic-ischemic injuries. Hepatocyte Growth Factor (HGF) and Vascular Endothelial Growth factor (VEGF) are the most important factors capable of inducing angiogenesis in areas that have undergone ischemic episodes and their importance is particularly relevant in wound healing. In cardiac regeneration, IGF-1 and VEGF mediate respectively an anti-apoptotic and angiogenic action, to which is attributed the capacity of ADSCs to have beneficial effects when transplanted/injected in different animal models of myocardial infarction/failure. In conclusion, most of ADSCs secreted factors act through mechanisms that mediate protection against cell death or, alternatively, induce cell migration and proliferation. Alternatively, they can indirectly act on the targeted cell populations: by promoting vascularization they can be indirectly linked to an increase of oxygen and nutrients in the affected areas, which may in turn promote local regenerative processes. Indeed, up to now most reports have focused on a limited set of known factors but it is expected that other molecules are responsible for the regenerative effects of ADSCs.

3.2.3. Immunomodulatory properties of ADSCs

The regenerative potential of ADSCs has been related also to their immune-modulatory abilities. ADSCs have been shown to be an immune-privileged site, preventing severe graft-versus-host response after transplantation procedures in vitro and in vivo. A concern of fundamental importance is the interplay between ADSCs and the host tissue, with particular focus on the immune system. Several studies have shown that ADSCs can be used either for autologous or allogenic cell transplants: this feature would be a major advantage for the employment of adipose tissue as a source for cell-based therapies. Furthermore ADSCs seem to act also as modulators of the immune system. The allogenic potential of these cells could be explained by the property of ADSCs to decrease the expression of hematopoietic markers and HLA-DR after subsequent passages. In addition, it has been observed that ADSCs only express HLA class I, but not HLA class II molecules: the latter can only be induced in ADSCs after incubation with IFN-γ. Furthermore, several experiments have proved that ADSCs do not stimulate lymphocyte proliferation and they do not elicit a response by Mixed Lymphocyte Reaction (MLR): in addition, they can also inhibit phyohemagglutinin (PHA)-stimulated lymphocyte proliferation. These immune-suppressive effects are likely mediated by soluble factors, among which PGE-2 seems to be the most important. Notably, the secretion of cytokines by ADSCs can be modulated not only by the inflammatory stimulus but also by the

surface upon which they are seeded: thus the bio-scaffold/environment provided could be another mechanism to control the immune-modulatory properties of ADSCs.

Main properties of ADSCs	
Differentiation potential	*Into cells of mesodermal origin*: adipocytes, fibroblasts, myocytes, osteocytes, condrocyes *Into cells of non-mesodermal origin*: endothelial cells, neuronal-like cells, pancreatic islet cells, hepatocytes
Secretion of soluble factors (ADSCs "secretome")	Adiponectin, angiotensin, cathepsin D, penetraxin, pregnancy zone protein, retinol binding protein, CXCL12, HGF, GM-CSF, ILs 6 ,7, 8, 11, TNF-α, VEGF, BDNF, NGF, GDNF, IGF-1, TGF-β, FGF-2, PGE2,
Immunomodulatory capabilities	Allogenic cell transplant potential Lack of response by MLR, Inhibition of PHA-stimulated lymphocyte proliferation

Table 3. Synopsis of properties of ADSCs.

4. Manipulation of adipose tissue and ADSCs

4.1. Introduction

Human subcutaneous adipose tissue provides an ideal alternative source of autologous pluripotent stem cells showing several advantages compared with other sources. As a matter of fact it is ubiquitous and commonly easily obtainable in large quantity with minimal invasive harvesting procedures or methods (either liposuction aspirates or subcutaneous adipose tissue fragments), limited patient discomfort and minimal ethical considerations: it may be transplanted safely and efficaciously. The abundance of stem cells available enables the direct therapeutic adoption of primary cells without any need for culture expansion. Moreover, adipose tissue is also uniquely expandable: currently available procedures for cell isolation yield a high amount of stem cells with remarkable properties of stable proliferation and potential differentiation in vitro, being attractive candidates for clinical applications offering protocols that may provide alternative therapeutic solutions in cell-based therapies and tissue engineering to repair or regenerate damaged tissues and organs. The technologies for adipose tissue harvesting, processing, and transplantation have substantially evolved in recent years together with appropriate commercial development and with updated refinements and information regarding extraction, isolation, storage, options for cultures, growth and differentiation, cryopreservation and its effect on survival and proliferation of isolated ADSCs, also related to their adoption in tissue-engineered constructs involving biomaterials and scaffolds. Inconsistencies in literature regarding the handling of ADSCs require more extensive investigations and controls, in particular in the in vitro processing and differences between the regenerative properties of freshly-processed heterogeneous stromal vascular fraction cells and

of culture-expanded relatively homogeneous ADSCs, or the related risk of complications and possible adverse events. There is a need for stronger evidence of the safety, reproducibility and quality of the ADSCs prior to a more extensive use in clinical applications. As a matter of fact, despite the clinical use of adipose tissue grafts and ADSCs worldwide has dramatically increased, questions concerning the safety and efficacy of these treatments are still opened and currently the use of isolated ADSCs for medical indications in a clinical setting has been approved only in selected cases and few countries.

4.2. Origins and delivery of adipose tissue grafts

Adipose tissue have been used for long time for reconstructive purposes through fat grafting or autologous fat transfer, a method according to which fat from the patient is removed from one area of the body ad reinserted into the desired recipient location. [9] Fat grafting has shown to be beneficial as a reconstructive and cosmetic procedure for patients with volume losses to soft tissues due to disease, trauma, congenital defects or aging. Even so, outcomes of these techniques are often unpredictable and rates of graft reabsorption may be disappointing. As a matter of fact, fat tissue is re-vascularized at the transplantation site within 48 hours from the surgical procedure, in the meantime being fed by diffused materials from surrounding free plasma. The survival rates of the graft are dependent on size of transplanted fat particles and on surface area from which these cells could re-establish their blood supply. In order to minimize reabsorption, studies have demonstrated the efficacy of less traumatic methods of harvesting, processing and injecting. Microinjection of fat by means of the "lipostructure technique" known also as Coleman's technique has been adopted by many plastic surgeons. [10] This technique distributes fat grafts in small aliquots by meticulous injection through multiple access sites, from which the graft fans out into various subcutaneous layers. The abundance of stem cells obtaina-ble in many common procedures, such as liposuction and liposculture, enables their direct therapeutic adoption without any need for culture expansion. [11] Even so, precursor cells can be purified by a variety of processes and enzymatic techniques may be adopted to obtain an ADSC-rich stromal vascular fraction (SVF). This issues are currently investigat-ed as adjuvants to free fat transfer in order to increase yield of graft retention (cell-assisted lipotransfer). The ADSCs contained in the stromal vascular fraction have been applied clinically as early as 2004 for the treatment of perianal fistulas in Crohn's dis-ease. [12] However, it is worth pointing out that, even though harvesting and first processing steps overlap, fat grafts SVF cells and ADSCs represent three different therapeu-tic options. Fat grafts are obtained directly after centrifugation of lipoaspirates. They contain predominantly mature adipocytes and are poor in ADSCs. The stromal vascular fraction, as mentioned above, is obtained by digestion with collagenase of the lipoaspi-rate sample and a subsequent centrifugation step: its cellular composition is heterogene-ous, being rich in ADSCs but containing also circulating blood cells, fibroblasts, pericytes and endothelial cells. The adoption of a pure ADSC population requires plating of the SVF and expansion of the stem cell population and thus, differently from the previous two options, ADSCs cannot be harvested and implanted in a one step-procedure. Even if all these approaches exploit to some extent the regenerative potential of adipose tissue they

are quite different procedures having also different therapeutic indications. Thus, attention has to be paid in order to avoid confusion. As for harvesting of ADSCs, several factors related to the patient, such as Body Mass Index (BMI) and age, have been analyzed for their impact on cell viability and number. Results are controversial, there is no evidence of a strong correlation of BMI with stem cells viability, number or size. Instead, there seems to be a negative correlation between age and rates of pre-adipocytes proliferation or differentiation, with higher lipolitic activity in the younger population and lower levels of apoptosis. [13] The body region of the donor site is another important variable patient-dependent. The abdomen, according to some studies, seems to be the best harvest site, while medial thigh and knee seem to have the lowest levels of viability of ASDCs. [14] These differences have not been proved in other studies. Effects of infiltration of local anesthetics during harvesting have also been investigated: lidocaine and adrenaline seem to have no effects on adipocyte viability. The method of harvest can affect not only viability of ADSCs but also their level of adhesiveness to extracellular matrix proteins. Standard liposuction allows the harvest of larger volumes of adipose tissue but it might result in up to 90% rate of adipocyte rupture. For this reason this technique is not ideal for fat grafting, while it could be more appropriate for ADSCs harvesting. An equivalent damage to pre-adipocytes has been measured comparing syringe aspiration with fat surgical excision. It is accepted that a larger cannula diameter at harvest correlates with improved cell viability. Partial purification of lipoaspirate can be carried out in the operatory room. The first step is centrifugation, which separates harvested fat into three layers: infra-natant (lowest layer composed of blood, tissue fluid and local anesthetics), middle portion (mostly composed by fatty tissue) and supra-natant (least dense upper layer including lipids). Infra-natant components can be ejected from the base of the syringe, while supra-natant can be poured off and soaked up using absorbent materials. While this technique is the most practical and today commonly used for fat grafting, it may not produce the best fraction of ADSCs possible. Several studies have been conducted on this issue, revealing that gentle centrifugation produces the highest cell viability, while long periods of centrifugation lead to isolation of the most proliferative cell type. When comparing decantation, washing and centrifugation, stem cells concentration results greater in washed lipoaspirates and pellets contained at the bottom of the centrifuged samples contain the highest concentration of stem cells.

4.3. Origins and delivery of ADSCs

Embryonic stem cells have an enormous multilineage potential but many ethical and political issues accompany their use. Therefore researchers have directed their attention on pluripotent adult stem cells. Adult stem cells were initially thought to have the differentiation capacity limited to their tissue of origin, however, as already mentioned above, many studies have now demonstrated that stem cells have the capacity to differentiate into cells of mesodermal, endodermal and ectodermal origin. MSCs from the bone marrow show extensive proliferative capacity and a multilineage differentiation potential into several lineages, including osteoblasts, chondrocytes, adipocytes and myoblasts. However, pain, morbidity and low cell numbers upon harvest represent an obstacle to their extensive clinical application. The

harvesting of adipose tissue, in comparison, is much less expensive than bone marrow. ADSCs can be isolated both from tissue samples and from lipoaspirate with less invasive procedures and are available in greater quantities (5 x 105 stem cells from 400 to 600 mg tissue). [15] ADSCs can be easily cultured and expanded, retaining their stem cell phenotypes and mesenchymal pluripotency still after several passages, features that make them an ideal source of stem cells for clinical applications.

• Isolation and culture of ADSCs

Since Rodbell's description of isolated pre-adipocytes from adipose tissue, a variety of methods have been developed. [16] Today, most laboratories use several common steps to process cells from adipose tissue. These methods include: washing, enzymatic digestion/mechanical disruption, centrifugal separation for isolation of cells which can be used directly, after cryopreservation, or after culture expansion for the generation of ADSCs. Still, despite the extensive use of ADSCs for research purposes, there is no any widely-accepted unique standard protocol for isolating and culturing these cells. For enzymatic digestion most laboratories use collagenases of different subtypes, trypsin, or a mixture of both, at various concentrations with an average incubation time of one hour, at 37°C, in constant shaking. The optimal centrifugation speed is considered to be around 1200g for 5 to 10 minutes. Some additional purification procedures can include filtration through nylon meshes and incubation with an erytrocyte-lysing buffer, usually Krebs Ringer Buffer (KRB) or NH4Cl. This procedure, however, seems to have a negative influence on the growth of ADSCs. Some investigators, after the identification of ADSCs surface immunophenotype, use immune-magnetic beads or flow cytometry to purify the stem cell population directly from the heterogeneous sample, using the CD34+ antigen. The most used culture medium are α-Modified Eagle's Medium (α-MEM), or Dulbecco's Modified Eagle's Medium (DMEM), after addition of fetal bovine/calf serum, (FBS/FCS), L-glutamine, penicillin and streptomycin.

• Cryopreservation of ADSCs

The development of simple but effective storage protocols for adult stem cells will greatly enhance their use and utility in tissue-engineering applications. [17] Cryopreservation is regarded as a promising technique and many studies have focused on this procedure. Other protocols investigated drying (anhydrobiosis) and freeze drying (lyophilization). The majority of in vitro studies agree that cryopreservation of adipocytes in liquid nitrogen, preferably using a set cooling and re-warming protocol, provides the lowest damage to cell viability. These results have been replicated in vivo (murine models) showing that grafts frozen in liquid nitrogen and stored at -35°C had a similar viability and histology compared to fresh tissue: in addition, this method obtained better results than freeze drying and immersion in glycerol. Recently, in order to increase the yield of adipose-derived stem cells post-thawing, the use of cryoprotective agents, such as dimethyl sulphoxide (DMSO) has been examined: samples frozen with DMSO achieved better outcomes than unprotected ones. Thus, cryoprotective agents are now considered as an essential part of any cryopreservation protocol aiming to provide appropriate conditions for the survival of ADSCs and adipocytes.

4.4. Safety concerns

Inconsistencies in literature regarding the handling of ADSCs require more extensive investigations and controls. In particular, a focus should be placed on in vitro processing as well as differences between the regenerative properties of freshly-processed heterogeneous adipose cells and those of culture-expanded relatively homogeneous ADSCs. Related risks of complications and possible adverse events like fat necrosis, seromas, oncological recurrences, should be accurately considered. In addition, adiponectin is implicated in the pathogenesis of insulin-resistant states, such as obesity and diabetes type 2. In particular, several studies reported that differentiated WAT cells and WAT resident progenitors may promote cancer growth and metastasis by means of a variety of different mechanisms (endocrine, paracrine, autocrine interactions). The main cellular component of WAT are adipocytes, the large cells accumulating tryglicerides in lipid droplets. In particular, in conditions like obesity, adipocytes in WAT may eventually became under oxygenated, leading to hypoxia, increased oxidative stress, recruitment of inflammatory leukocytes and eventually fibrosis. In recent experimental models, some adipokines showed to be able to promote tumor growth along with fatty acids released by adipocytes. High levels of adiponectin have been associated with the development of endometrial carcinoma and breast cancer. Leptin has been identified in regulation of cell proliferation and neo-vascularization in malignant and normal cells of different origins, including lung, gastric, colonic, kidney, leukemic, hematopoietic and epithelial cells. Notably, these molecules can enhance proliferation and survival of malignant cells and/or of tumor vasculature. So far, studies investigating the role of WAT in cancer have predominantly focused on pro-tumorigenic effect of ADSCs. In fact the increased proliferation and survivor of malignant cells may result from the engagement of perivascular ADSCs into angiogenesis and vascular maturation, resulting in improved tumor blood perfusion. Cytokines such as adiponectin, leptin, interleukin-6, and TNF alfa seem to be responsible for a chronic low-grade inflammation. Furthermore, mesenchymal cells are known to suppress the activation of T-killer cells: this finding suggests that also ADSCs may help tumors to evade the host immune response. Thus, adipocytes may be able to produce adipokines and several secretions which could potentially induce cancer reappearance by "fueling" dormant breast cancer cells in tumor bed true "tumor-stroma interaction": even so, up to now, especially for grafting of adipose tissue after breast cancer treatment, there is no strong clinical evidence or international agreement on this topic. [18-19] Depending on country, the safety of adipose tissue grafting is still a controversial issue. In 2009, the American Society of Plastic surgeons Fat Graft task Force concluded that no reliable studies could confirm definitely the oncologic safety of lipofilling in breast cancer patients. A more accurate point of view is provided by a large multicentric observational study on adipose tissue grafting in patients previously affected by breast cancer: considered parameters included the complication rate of the technique, the risk of modification of mammography and a rigorous long-term clinical/instrumental follow-up. [20] At the moment no studies on the effects of lipotransfer on human cancer breast cells in vivo are available. We cannot provide the definitive proof of the safety of lipofilling in terms of cancer recurrence or distant metastasis, but until then, should be performed in experienced hands, and a cautious oncologic follow-up protocol is advised.

5. Clinical use

5.1. The regenerative cells

The growing interest in this area of research has driven the adoption of adipose tissue and ADSCs in a wide number of clinical situations, medical fields and conditions for the repair and regeneration of acute and chronically damaged tissues, with an increasing number of translational efforts. Clinical trials have been advanced in order to investigate the therapeutic potential and applicability of these cells based on the induction of their properties similar to that observed in BMSCs. An extensive great knowledge concerning the harvesting, characterization and transplantation of ADSCs has been developed. Even so, current literature still lacks of strong evidence about the clinical potential of ADSCs and adipose tissue. In particular this may be due to the fact that human lipoaspirates may significantly differ in purity and molecular phenotype and that many reports have adopted heterogeneous populations of cells providing uncertain results. Remarkably, some problems still affect the correct interpretation of outcomes. One of the most significant issues limiting the interpretation of clinical progression is the lack of standardization in defining ADSCs, since both SVF and ADSCs may be used. [4] Another issue is whether ADSCs operate on tissue regeneration through direct transdifferentiation or paracrine mechanisms based on the secretion of numerous cytokines and growth factors. Thus, standardization of a method and improvement of current preclinical data may allow direct comparison of different results as well as a better definition of clinical potential of ADSCs. Current preclinical and clinical data of such cell-based therapies should include the osteogenic, chondrogenic, adipogenic, muscular, epithelial and neurogenic differentiation of progenitor, endothelial, and mesenchymal stem cells involved. Thus, skin, bone, cartilage, muscle, liver, kidney, cardiac, neural tissue, pancreas represent some of the most prominent clinical targets on which these therapies are focused. ADSCs are commonly adopted in clinical settings in surgical fields such as: cell-enriched lipotransfers, soft tissue augmentations and reconstructions of defects after trauma or oncologic surgery, healing of chronic wounds (phase 1 trials for the healing of recurrent Crohn's fistulae), skin regeneration and rejuvenation (repair of damages induced by aging or radiations), scar remodeling. In addition, they have been adopted in the treatment of cardiovascular disease, metabolic disease and encephalopathy (cerebral infarction) and a wide range of other surgical needs by orthopedic surgeons, oral and maxillofacial surgeons and cardiac surgeons. Indeed, the clinical application of adipose tissue relies on convincing results but the full therapeutic potential of ADSCs may still need further investigation.

5.1.1. The "Lipofilling technique"

Fat graft has been initially adopted to generate adipose tissue in the treatment of contour deformity or volumetric defects. The "lipofilling technique" has been used for many years and it has become rapidly popular especially in aesthetic surgery to improve cosmetic results in facial surgery. In fact it may be considered an ideal filler since it is totally biocompatible, readily available, inexpensive and it enables good aesthetic results. More variable are the application of fat injection in reconstructive surgical treatments. For example in breast reconstruction the

indication of lipofilling include micromastia, tuberous breasts, Poland syndrome, post-lumpectomy deformity, post-mastectomy deformity, sequelae of post-radiotherapy (every anatomical region previously subjected to radiotherapy is subject to fat injection), refinement of secondary reconstructions after flap or prosthesis reconstruction and nipple reconstruction. In head and neck reconstructive surgeries it has been used to correct Treacher Collins syndrome o other cranio-synostosis. In burns, lipofilling has been adopted to improve the structural features of extracellular matrix in the treatment of burn sequaele, such as pathologic scars, with the aim to restore a more physiologic skin architecture. The lipofilling is also a valuable option to enhance volumes in facial hypotrophies, for example in patients affected by HIV-related lipo-distrophy. In addition, fat injection has proved to be very useful to improve local vascularization and trophism in chronic ulcers, especially vascular or post-traumatic ulcers.

Figure 2. Injection of autologous adipose tissue ("lipofilling technique") in a scar.

5.1.2. Clinical trials with ADSCs

Most of clinical trials on humans are based on previous experiments on animal models. The evidence of the ability of ADSCs to differentiate into cells of non-mesodermal origin has been tested in some models in treatment of several diseases. The ADSC-derived hepatocytes transplanted into nude mice restored liver function and freshly isolated ADSCs could differentiated into hepatocytes after intrasplenic transplantation into nude mice in vivo, supporting their application in clinical setting. [21] However clinical trials are still mostly lacking of

promising results. [4] A recent study showed that the direct injection of ADSCs could restore blood flow in a mouse ischemic hindlimb model, as confirmed by clinical data. [22] The myogenic differentiation of ADSCs may be used in the treatment of muscular diseases such as Duchenne dystrophy and for regenerative cell therapy in heart failure. [23] Other novel potential clinical uses of ADSCs include the treatment of Alzheimer disease, of multiple sclerosis due to the anti-inflammatory effect of ADSCs, of neurogenic bladder and other neurologic disorders. A preliminary study showed that peri-urethral injection of autologous ADSCs acts positively in stress urinary after prostatectomy. Regarding current clinical applications of ADSCs, apart from a phase III trial on the treatment of Crohn's fistula, most clinical trials are in phase I. Beside the use in breast reconstruction, trials are in progress to treat acute myocardial infarction and chronic myocardial ischemia by intracoronary injection of SVF. Other trials are focused on the treatment of cirrhosis and of diabetes I or II. [4] Another trial adopted ADSCs (after purification and expansion) for the management of fistulas associated or not to Crohn's disease: results demonstrated an efficient control of inflammation and an improvement of healing process, most likely due to paracrine action that cells differentiation. Another trial investigated the restoration of volumes in hypotrophic scars after subcutaneous injection of ADSCs. Only two trials have studied the effect of ADSCs on chronic critical limb ischemia: the first adopting intra-muscular injection, the second by intravenous injection in diabetic patients. The literature regarding different clinical trials [Table 4.]demonstrates that ADSCs-based therapies are a concrete opportunity but despite these results, molecular, cellular e biological features of these cells are still uncertain and it is also unclear if regenerative therapy is related to their differentiation potential or paracrine activity: indeed, more appropriate in vivo investigations are necessary.

Pathology	Operating methods	Condition
Stress urinary after prostatectomy	peri-urethral injection of autologous ADSCs	Report of three initial cases
Crohn's fistula	injection into rectal mucosa of autologous of ADSCs with fibrin glue	Phase III
Cirrhosis	intrahepatic arterial administration of autologous SVF	Phase I
Diabetes I	intravenous injection of autologous SVF	Phase I/II
Diabetes II	autologous SVF	Phase I/II
Hypotrophic scars	subcutaneous injection of ADSCs	Phase III
Chronic critical limb ischemia	intra-muscular injection of ADSCs	Phase I
Chronic critical limb ischemia in diabetic patients	intravenous injection of ADSCs	Phase I/II
Myocardial infarction	intracoronary injection of SVF	Phase II/III
Multiple sclerosis	intravenous injection of autologous ADSCs	Phase I/II
Reumathoid arthritis	intrarticular injection of autologous ADSCs	Phase III

Table 4. Clinical trials using adipose-derived stem cells (ADSCs) or stromal vascular fraction (SVF).

6. Tissue engineering

6.1. Adipose derived bio-products

In the past decade, preclinical and translational efforts have established the future basis for the application of ADSCs from the bench to the bedside. Significantly, ADSCs have been widely used in tissue engineering, organ repair and gene therapy. These multipotent cells have shown a remarkable plasticity and the ability to differentiate towards different cell lineages with similar yet enhanced properties (their multipotency and proliferative efficiency) in comparison to bone marrow-derived mesenchymal stem cells. [3,6-7,21-24,26] Moreover, ADSCs also show adjuvant angiogenic properties likely related to the secretion of vascular endothelial growth factor. [21] In vitro studies have rapidly increased during the last decade, resembling the need to optimize the variables of the differentiation process cells towards the desired lineage. The efficient use of biomaterials, delivery vehicles and bioreactors has promoted the development of a large variety of novel tissue engineered products for repair and regeneration of various tissues and organs. The use of suitable animal models in an extensive preclinical literature has also established the basis for successful stem cell-based therapies that may implement current therapeutic solutions for several diseases. Thus, a focus of most interest for the scientific community is posed today in the production of safe and reliable cell delivery vehicles/scaffolds useful in applying ADSCs as a therapy as well as in the development of novel suitable in vivo animal models. A large variety of bioengineered products have been developed by means of selected differentiating cultures of ADSCs. [Table 5] Preclinical studies have experimentally reported the adoption of ADSCs in order to develop cells of mesodermal origin as well as cells of non-mesodermal lineage such as neural o neural-like cells for repair of neural traumatic injuries, fibroblast for reconstruction of soft tissue defects, tenocytes or regenerated tendon constructs for optimal musculoskeletal system reconstruction, osteoblasts for bone tissue replacement, chondrogenic lineages and cartilage substitutes for implantation, skeletal muscle cells and subsequent myotube-like formation depicting myogenic differentiation in vivo in muscular dystrophy model. Other reported lineages and engineered tissues that may be obtain through selective differentiation include hepatocytes, pancreatic endocrine cells, cardiomyocytes and vascular endothelial cells. [24] Most relevant transcription factors involved in differentiation into adipocytes, chondrocytes, myocytes and osteocytes are well-known. However, in addition to specific differentiation factors, tridimensional biomaterials are essential to address differentiation of ADSCs to the required cell type and to use them for tissue-engineering purposes. Among investigated effective scaffolds and matrices we may include: type I collagen, hyaluronic, poly lactic-co-glycolic acid (PLGA) and silk fibroin-chitosan. [26] Moreover, the combination with specific growth factors determines the overall outcome of the applied biopolymer.

Tissue	Cell type	Gene	Scaffold	Result
Bone	human ADSCs	BMP-2	-	heal critical sized femoral defects in a nude mouse model
	ADSCs	BMP-2	collagen sponge	increase bone induction in SCID mice
	Autologous SVF	-	bone graft	treat calvarial defects in human
	Autologous ADSCs	-	β-tricalcium phosphate-filled titanium scaffold	create neo-maxilla in human
Cartilage	ADSCs	-	polyglycolic acid scaffolds	exhibit in vitro chondrogenic characteristics
	ADSCs	-	-	improve outcome measures in osteoarthritis in dogs
Endothelia	ADSCs	-	porous polycaprolactone (PCL) scaffold	endothelial differentiation
Tendon	ADSCs	-	decellularized human tendon	recellularize
Nerve	ADSCs	-	hyaluronan membrane and fibrin meshes	differentiate in glial-like and neuronal-like cells

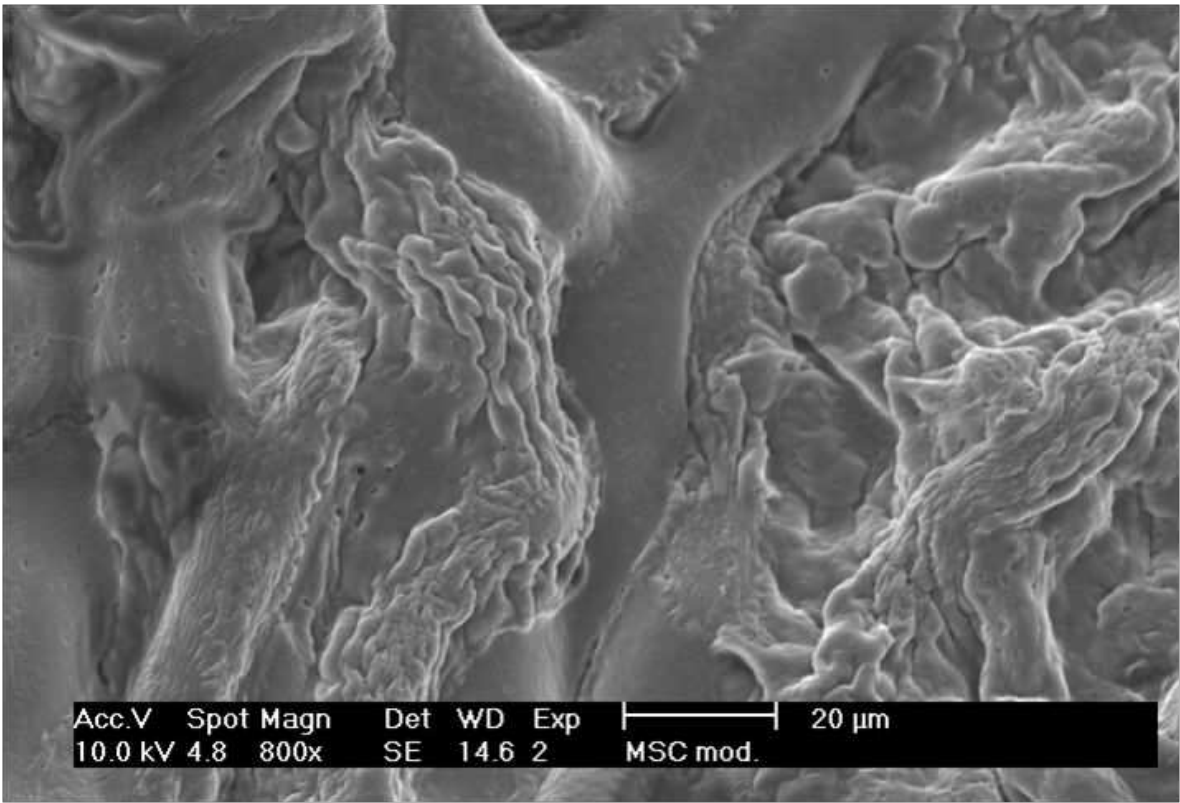**Table 5.** Synopsis of current approaches in ADSCs and tissue engineering.

Figure 3. Electron microscopy scanning of ADSCs cultured on a Hyaluronic acid-based biomaterial.

6.1.1. Bio-engineered bone

There is still a clinical need to generate bone for the repair of large osseous defects, since current strategies are based on non-vascularized bone grafts, suitable only for small defects. As an alternative, progenitor cells might be implanted on biomaterials and differentiated in vivo supporting reconstruction of large bone losses. Osteo-inductive factors include vitamin D3, β-glicerophosphate, acid ascorbic and Bone Morphogenic Proteins (BMPs). [7] Treating ADSCs with recombinant BMP-2 has shown to stimulate osteogenic differentiation: [27] human ADSCs overexpressing BMP-2 could heal critical sized femoral defects in a nude mouse model. Similarly, ADSCs exposed to BMP-2 adenoviral transfection and seeded in collagen sponges increased bone induction in SCID mice. [27-28] These results suggest that transfected stem cells can replace the exogenous addition of growth factors when transplanted in a bio-engineered scaffold. The use of scaffolds is critical in repair of structural tissues such as bone. Demineralized bone matrix, collagen, PLGA, hydroxyapatite and β-tricalcium phosphate scaffolds were reported to be suitable for ADSC-derived osteochondral tissue engineering. Most of clinical trials of osteogenesis in ADSCs rely on murine studies and human trials are based on very limited reports. The first human case involved transplantation of SVF together with bone graft to treat calvarial defects [29] and in another case a neo-maxilla has been created using a β-tricalcium phosphate-filled titanium scaffold associated to cultured ADSCs. [30] Thus, ADSCs-based osteogenesis is possible, however, more adequate evidence is needed in the clinical setting.

6.1.2. Bio-engineered cartilage

ADSCs might be used to generate cartilage for clinical use in the treatment of degenerative joints. The list of potentially useful growth factors for cartilage repair comprises TGFβ, IGF-1, FGFs, EGF and BMPs, transcription factors as SOX9 and signal transduction molecules such as SMADs. Several in vitro studies have shown the chondrogenic differentiation of ADSCs and this feature is confirmed by their ability to generate cartilage in a variety of experimental models. ADSCs seeded into polyglycolic acid (PGA) scaffolds exhibited in vitro chondrogenic characteristics and they could synthesized cartilage extracellular matrix. [23] The great potential of ADSCs in cartilage tissue engineering was also demonstrated in different studies in vivo. Moreover ADSCs have been used recently for treatment of osteoarthritis in dogs [32] and rheumatoid arthritis in human. [33] However, given the lack of evidence, it seems likely that the symptomatic benefits seen in these trials may relate to the anti-inflammatory properties of ADSCs rather than to a real chondrogenic differentiation.

6.1.3. ADSCs and vascular/endothelial tissue engineering

The vascularization of regenerated tissues is an important field of research since it allow the survival of tissue and the differentiated cells. [24] It has been reported that human ADSCs have the potential for endothelial differentiation and they can participate in blood vessel formation by means of the secretion of several pro-angiogenic factors, like vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). [23] This feature makes these cells suitable for regenerative cell therapy, treatment of ischemic disorders and construction of

vascularized grafts in one-step procedure, as it has already been performed in many experiments on animal models. [22] Furthermore, as reminded, the angiogenetic properties of ADSCs have been already investigated in several clinical trials to treat various diseases.

6.1.4. Bio-engineered tendon

Tendon tissue engineering is relatively unexplored due to the difficulty to maintain in vitro preservation of tenocyte phenotype: only recently research has demonstrated the fundamental role of in vitro mechanical stimuli in maintaining the phenotype of tendinous tissues. [34] The main growth factors inducing tendon differentiation include fibroblast growth factor (FGF), platelet-derived growth factor-BB (PDGF-BB), epidermal growth factor (EGF), insulin-like growth factor (IGF)-1 and members of the transforming growth factor-β (TGF-β)/bone morphogenetic proteins (BMPs) family. Several in vivo and in vitro studies have showed the ability of ADSCs to differentiate in tenocytes under specific stimuli and under biomechanical force. [34] Furthermore, recent experiments have focused on the possibility of re-cellularize by means of seeded ADSCs a decellularized human tendon. [35] Thus, an integration of ADSCs, growth factors, mechanical stimuli and biopolymers may provide a solution for the treatment of difficult tendon injuries

6.1.5. ADSCs and neuronal tissue-engineering

Incubation of ADSCs under neuro-inductive conditions (culture medium containing EGF, FGF, NGF and BDNF) has shown the potential to form neurospheres expressing neurospecific markers, including nestin, βIII tubulin, S100 and glial fibrillar acidic protein (GFAP). [36] Moreover, seeding of these neurospheres in different scaffolds (hyaluronan based membranes and fibrin glue meshes) demonstrated further differentiation in glial-like and neuronal-like cells. [37] Although these are only preliminary researches, these promising results are of significant clinical interest. ADSCs-induced neural cells may provide beneficial therapeutic effects in treatment of injuries occurring to both the peripheral and central nervous systems such as in the treatment of neurodegenerative states, including Parkinson's disease, Hungtinton's disease, multiple sclerosis and Alzheimer's disease.

7. Prospectives

Regenerative medicine is an evolving field of research and therapeutics in which adipose tissue and ADSCs hold great promise for translational research and future clinical applications in many fields of tissue regeneration with a wide range of potential clinical implications. In the past decade, preclinical data from in vitro studies and pre-clinical animal models has been provided on the reproducibility, safety and efficacy of ADSCs in tissue regeneration or tissue engineering, supporting their use in clinical applications and establishing the basis for a translational application in the bedside: consistently, recent preliminary clinical trials have confirmed positive outcomes. The enhancing effect of ADSCs on autologous repair might enable better clinical outcomes and play a relevant role in healing acute and chronic tissue

damage. Thus, more accurate information regarding optimal management and methods to promote differentiation lineages (among which differentiation factors, cell scaffolds, cell culture conditions) are strongly required. Further translational research, adequate clinical investigation and novel strategies should be promoted and designed to overcome current limitations, encourage future therapeutic implementation and face challenges posed by regenerative medicine.

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