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Human Adipose Stem Cells: From Bench to Bed-Side

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Stem cell-based therapies for repair and regeneration of different tissues are becoming more important in the treatment of several diseases. Adult stem cells currently symbolize the most available source of cell progenitors for tissue engineering and repair and can be harvested using minimally invasive procedures. Moreover, mesenchymal stem cells (MSCs), the most widely used stem cells in stem cell-based therapies, are multipotent progenitors, with capability to differentiate into cartilage, bone, connective, muscle, and adipose tissue. So far, bone marrow has been regarded as the main source of MSCs. To date, human adult adipose tissue may be the best suitable alternative source of MSCs. Adipose stem cells (ASCs) can be largely extracted from subcutaneous human adult adipose tissue. A large number of studies show that adipose tissue contains a biologically and clinically interesting heterogeneous cell population called stromal vascular fraction (SVF). The SVF may be employed directly or cultured for selection and expansion of an adherent population, so called adipose-derived stem cells (ASCs). In recent years, literature based on data related to SVF cells and ASCs has augmented considerably: These studies have demonstrated the efficacy and safety of SVF cells and ASCs *in vivo* in animal models. On the basis of these observations, in several countries, various clinical trials involving SVF cells and ASCs have been permitted. This review aims at summarizing data regarding either ASCs cellular biology or ASCs-based clinical trials and at discussing the possible future clinical translation of ASCs and their potentiality in cell-based tissue engineering.

Introduction

In recent years, stem cells have been contemplated to be a novel option in clinical therapy regarding tissue formation and regenerative medicine with the utilization of biomaterial scaffolds and growth factors.

Stem cells can be defined as undifferentiated cells with a capacity of self renewal and differentiation into multiple lineages.[1] In contrast to embryonic stem cells, stem cells that arise from differentiated tissues are immuno-compatible and are not subject to ethical challenges.[2,3] Mesenchymal stem cells (MSCs) isolated from adult tissues have proved, so far, to be the most suitable for clinical applications. Bone marrow is considered an important site for hematopoietic stem cells and is, additionally, the main source of MSCs as a part of its stromal fraction.[4–7] MSCs serve as a small, nonhematopoietic committed subpopulation of cells located in the bone marrow, already reported by Friedenstein *et al.*[8] These multipotent cells are easily isolated from bone marrow and can expand and differentiate into mesoderm-derived lineages such as adipocytes, chondrocytes, osteoblasts, and myoblasts[9–12] as well as into neuro-ectodermal-derived lineages such as neurons.[13] These observations gave rise to the birth of MSC-based therapies: Clinical trial investigations using bone marrow-derived MSCs to treat debilitating conditions such as osteogenesis imperfecta,[14] myocardial infarction,[15] and strokes[16] have shown some therapeutic promises. However, the clinical use of bone marrow MSCs has raised several issues worthy of addressing, including the procedure of bone marrow harvest with related pain, side effects, and morbidity. To address these problems, many researchers have investigated alternative sources of MSCs and recently new sources have been discovered in the following tissues: dental pulp,[17,18] Wharton jelly,[19] amniotic fluid,[20] and adipose tissue.[21] Adipose tissue, as well as bone marrow, originates from the mesenchyme and contains a supporting stroma that could be readily available for isolation. Based on this observation, studies have reported that adipose tissue may be an important source of MSCs with far-reaching effects in various

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fields. MSCs may be largely isolated from adipose tissue and in culture is able to display stable growth and proliferation kinetics.[22,23] Adipose stem cells (ASCs), as in bone marrow MSCs, can differentiate *in vitro* into adipogenic, osteogenic, chondrogenic, and myogenic cells when cultured in specific lineage-inducing culture media[24–27] and into endothelial cells.[28]

In conclusion, the high abundance of adipose tissue within the body, its high surgical accessibility, and the demonstrated multi-potency of ASCs show adipose tissue as a promising candidate for MSCs harvest and increase the interest in its use in tissue repair, regenerative medicine, and degenerative disease management. The aim of this review is to summarize the body of literature and the data regarding this topic.

Isolation, Amplification, and Advantages of MSCs Derived from Adipose Tissue

As reported by the International Federation for Adipose Therapeutics and Science (IFATS),[29] ASCs seem to be ideal in regenerative medicine applications for many reasons: They can be harvested, handled, and increased in number in a noninvasive, easy, and effective manner.

In the 1960s, Rodbell *et al.*[30] were pioneers of the first methods for ASCs isolation. Subsequently, this procedure was modified by Zuk *et al.* in 2001[24] to allow stem cell isolation from human adipose tissue specimens. There may still be discrepancies regarding the results from laboratories due to the differences in methods used. To overcome this problem, Nicoletti *et al.* set a new standard protocol to isolate ASCs from human adipose tissue using enzymatic digestion[31] (Fig. 1).

The great novelty of this protocol is that cells isolated from adipose tissue were sorted by fluorescence-activated cell sorting before plating (see Characterization of ASCs: Immunophenotype and Immunomodulatory Properties): This protocol guarantees a greater percentage of ASC purity and viability. Moreover, this novel procedure has been used by other groups to select a specific cell subpopulation characterized by a specific immunophenotype (see Characterization of ASCs: Immunophenotype and Immunomodulatory Properties).[32,33] Nonetheless, it is still necessary to establish one standardized protocol to isolate ASCs to be used in clinical practice, providing more homogeneous and consistent results in regenerative medicine. We may also consider the important clinical implications of adipose tissue auto grafting in patients affected by breast cancer. Using a single standardized protocol would facilitate follow up and permit comparisons between different institutions that operate autologous transplantation of adipose tissue.

Characterization of ASCs: Immunophenotype and Immunomodulatory Properties

In 2002, Zuk *et al.* reported that ASCs expressed multiple clusters of differentiation (CD), similar to those observed in bone marrow MSCs.[21] It is worth mentioning that Gimble *et al.* noted that adhesion substrates and culture time modify the differentiation status of ASCs: In particular, after two passages in culture, the ASCs show peculiar adhesion and receptor molecules, surface enzymes, extracellular matrix (ECM), and proteins that are correlated to the stromal cell phenotype.[34] Different groups performed direct comparisons between human ASCs (hASCs) and bone marrow MSCs. Data indicated by Zuk *et al.* reported that these two stem cells were immunophenotypically 90% identical.[21] More recent papers have revised this previous statement, giving a different panel of molecular identity.[35] For instance, the glycoprotein CD34 is evident on hASCs at the first passage but is not present on bone marrow MSCs[4] (Table 1). It is worth mentioning that the presence of Stro-1 antigen, a common bone marrow MSC-associated surface antigen, on hASCs is a matter of debate.[21,36] More recently, Sca-1

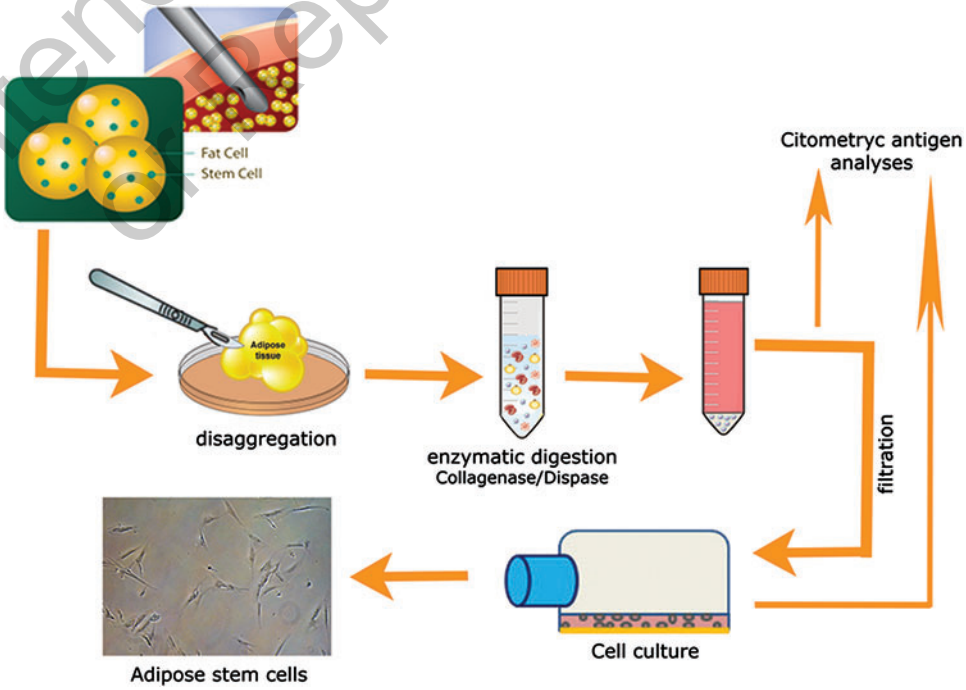

FIG. 1. Schematic representation of adipose stem cells (ASCs) isolation protocol described by D'Andrea *et al.*[85] in 2008. Adipose tissue obtained by lipectomy or liposuction was washed, mechanically minced, and digested in collagenase type I and dispase solution. Digested tissue was filtered and centrifuged. Erythrocytes were eliminated, and the remaining cells were cultured in Dulbecco's modified Eagle's medium 10% fetal bovine serum on plastic dishes. After 3 days of culture, adherent cells acquired a fibroblast-like or polygonal shape and the cells were characterized cytomethically to establish the stemness degree of this cell population. Color images available online at www.liebertpub.com/teb

Table 1. Cluster of Differentiation

CD	*ASCs*	*BM-MSCs*
CD9	++	+
CD10	++	±
CD11a	—	+
CD11b	—	+
CD11c	—	+
CD13	++	++
CD14	—	—
CD16	—	+
CD18	—	+
CD29	++	++
CD31	±	+
CD34	+	–
CD44	++	++
CD45	—	–
CD49b	±	++
CD49d	±	++
CD49e	++	++
CD50	—	+
CD51	++	++
CD54	±	+
CD55	++	++
CD56	—	+
CD59	++	++
CD61	±	±
CD62e	±	+
CD63	±	±
CD71	±	+
CD73	+	++
CD90	++	++
CD104	—	±
CD105	++	++
CD106	±	+
CD117	–	++
CD133	±	++
CD140a	±	+
CD146	±	++
CD166	+	+

++, >70%; +, 30–70%, ±, 10–30%; –, 2–10%; —, <2%; ASC, adipose stem cell; BM, bone marrow; CD, clusters of differentiation; MSC, mesenchymal stem cell.

surface marker has been found in a cell subpopulation derived from white adipose tissue enriched in stem cells.[37,38] Pinpointing of the ASC surface immunophenotype has allowed the possibility to improve or to refine the stem cell population from the heterogeneous stromal vascular fraction (SVF) cells. Scientific researchers have used immune-magnetic beads or flow cytometry for positive and negative selection of a cell subpopulation within the SVF. In particular, Miranville *et al.*[39] and Suga *et al.*[40] selected the $CD34^+/CD31^-$ cell subpopulation also expressing CD133 and ABCG2. This population exhibited characteristics of endothelial progenitor cells. Moreover, Mitchell *et al.*[41] characterized SVF behavior *in vitro* showing that at the outset, stromal cell-associated markers (CD13, CD29, CD44, CD63, CD73, CD90, CD166) had fewer SVF cells increasing significantly in successive passages, whereas stem cell markers (CD34 and ABCG2) subsequently decreased. Traktuev *et al.*[42] detected the $CD34^+/CD31^-/CD144^-$ cell subpopulation at peri-vascular level in adipose tissue. This population showed smooth muscle (α-actin, caldesmon, and calponin), pericytic (chondroitin sulfate proteoglycan, CD140a, and CD140b), and mesenchymal (CD10, CD13, and CD90) markers. Finally, De Francesco *et al.*[28] isolated a cell subpopulation characterized by positivity for CD29, CD34, CD44, CD90, CD105, and CD117 from SVF. This cell population differentiated both *in vitro* and *in vivo* into endothelial cells, resulting in the formation of capillary-like structures in a methylcellulose medium. Interestingly, Sengenes *et al.*[43] highlighted that the CD34-positive SVF cell population included only the ASCs population. Despite numerous studies that investigate the different markers recommended to detect and isolate ASCs, there is no unanimous consensus in using a specific combination of CDs that may select MSCs from adipose tissue. The characterization of biological behavior using other stemness tests is fundamental. For example, the expression of transcription factors such as *OCT4*, *SOX2*, and *NANOG* that are involved in maintaining stem cells; the ability to proliferate; self-renewal; and the differentiation potential are key factors to be considered.

ASCs and bone marrow MSCs are immuno-privileged and immune-tolerated in auto-graft *in vivo*. Several studies have indicated that both ASCs and bone marrow MSCs inhibit peripheral blood mononuclear cell proliferation,[44,45] and ASCs show a higher level of secretion of cytokines with potent immunomodulatory effects such as interleukin-12 (IL-12), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ).[46] These data suggest that ASCs, as well as bone marrow MSCs, may be considered in allogenic regulation[47] and may be used to reduce graft-versus-host diseases as reported by Fang *et al.*[48]

ASCs *In Vitro* Cell Potency

The multi-potency of ASCs, such as bone marrow MSCs, plays an important role in mesodermal defect repair and disease management. In particular, both ASCs and bone marrow MSCs have demonstrated the ability to differentiate into adipogenic,[49,50] osteogenic,[51,52] chondrogenic,[53,54] endothelial cells,[55,56] and the myogenic[57,58] lineage. Noteworthy, ASCs and bone marrow MSCs have also demonstrated their competence to differentiate into other tissues such as neuron-like cells[59,60] and epithelial cells.[61,62]

Adipogenic lineage

In reconstructive plastic surgery, adipose tissue is frequently a key factor in tissue loss or defect.[63] In recent years, fat grafting may not only depend on transplantation of adult adipocytes but may also rely on transplantation of the ASC fraction.[64,65] Growth hormone, glucocorticoids, insulin, and prostaglandins are stimulatory for ASCs in adipocyte differentiation in the initial and final stages. Consequently, the adipogenic media are usually supplemented with the second messenger cyclic adenosine monophosphate (*cAMP*) inducer 3-isobutyl-1-methyl-xantine (IBMX), and dexamethasone, human recombinant insulin, and indomethacin. These substances activate gene expression involved in adipogenic differentiation and inhibit the expression of those genes that aid in maintaining stemness and/or in the differentiation process of other tissues. The adipogenic conditions that have an impact on ASCs are necessary to acquire the morphology specific to lipid-laden cells evidencing intracellular lipid

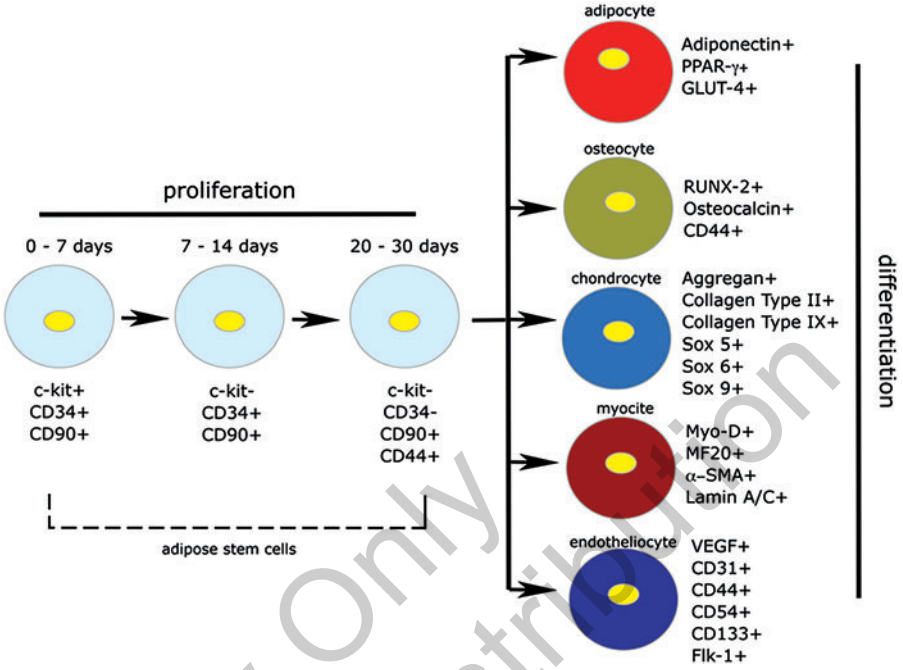

FIG. 2. Schematic representation of ASCs proliferation and differentiation potency. Color images available online at www.liebertpub.com/teb

droplets, which exhibit adipocyte marker expression such as *adiponectin* and peroxisome proliferator-activated receptor gamma (*PPARγ*) even after 24 h (Fig. 2).

The production *in vitro* of adipose tissue is frequently required in reconstructive and cosmetic surgery. The capacity of ASC to differentiate into mature adipocytes is clearly evident in the exposure medium containing steroids, a *cAMP* inducer, and fatty acids. The differentiation of ASCs into adipocytes is unquestionable, and the discovery of ASC as a regenerating source of adipocytes for fat grafting is of great interest. For this purpose, many clinical trials are set up with the aim of improving the results of fat grafting for contour deformities or breast augmentation. Interestingly, fat grafting is already largely involved in the practical application of ASC, a procedure with a positive clinical response.

Osteogenic lineage

In clinical practice, differentiation of ASC into osteoblasts might be useful in repair and regeneration of fractures, and it may assist bone grafting of join fusions.[32,66] In 2003, Lee *et al.* reported early demonstrations of *in vivo* bone reconstruction after *in vitro* differentiation of ASCs toward the osteoblastic lineage.[67] In 2004, Cowan *et al.* showed that ASCs, implanted onto apatite-coated scaffolds, were able to heal critical-sized mouse calvarias defects.[68] There is agreement in the scientific community regarding the requirement of dexamethasone and 1,25-dihydroxyvitamin D3 for *in vitro* osteogenesis induction.[21,27] Ascorbic acid addition is also important, because this coenzyme works as a co-factor in the hydroxylation of lysine and proline residues in collagen, accelerating the synthesis of noncollagenous bone ECM proteins. Moreover, β-glycerophosphate is necessary to calcify and mineralize bone ECM (Fig. 2).

It is also important to generate bone to repair large bone defects, in a clinical context. Traditionally, no vascularized bone grafts may be retained adequate to repair bone defects that are lesser than 6 cm, while vascularized bone grafts are required to heal bigger areas. However, there is evidence that ASCs differentiation toward the osteogenic lineage is more complex than the adipogenic differentiation. Staining for calcified ECM components such as Alizarin Red may be a simple procedure. The markers commonly assessed to demonstrate osteogenic differentiation are *RUNX2*, *osteocalcin*, *osteopontin*, *type I collagen*, and *ALP*. There are few clinical trials regarding osteogenesis using ASCs. Bone differentiation from ASCs is clearly possible, but developing clinically relevant volumes and length of bone *in vitro* may remain an arduous task.

Chondrogenic lineage

ASCs could be used to treat traumas or arthritis in joints and may be useful in joint reconstruction. ASCs have been employed in rabbits for the repair of osteochondral defects.[69] Chondrogenic differentiation is induced *in vitro* by insulin, transforming growth factor-beta 1 (TGF-β1), and ascorbat eaddition without dexamethasone to the medium. Enhanced expression of cartilage ECM proteins takes place when a three-dimensional (3D) culture technique is used to suspend ASCs.[70] When cells grow as a monolayer, the chondrogenic markers of differentiation are seen; however, in reduced concentration and cellular differentiation, they appear incomplete[71] (Fig. 2).

ASCs are expected to positively generate cartilage for clinical use in the treatment of joint degenerative diseases. The use of cartilage regeneration instead of joint replacement may be an alternative treatment with socioeconomic advantages. A simple procedure used to demonstrate ASCs differentiation into a chondrogenic lineage is staining for increased expression of proteoglycans using Alcian Blue or Safranin-O. Genes frequently used for chondrogenic differentiation include *type I collagen*, *type II collagen*, *cartilage oligomeric matrix protein*,

and *aggrecan core protein*. Considering the little evidence, to date of structural improvement in the cartilage of the joint, one may deduce that the symptomatic benefits observed in these trials are linked to the anti-inflammatory properties of ASCs rather than to the condrogenic differentiation. There is poor evidence of cartilage reconstruction by ASC; therefore, new protocols are required to obtain results.

Endothelial differentiation

The ability of ASCs in supporting angiogenesis is a matter of debate, most likely because the cell population isolated by the different scientific groups is not homogeneous and the percentage of different cell precursors may undergo slight alterations in the different laboratories. The first studies on ASCs differentiation came to the conclusion that endothelial cells were the most represented cell type in the SVF, as indicated by the presentation of endothelial cell-specific markers such as CD31, CD44, CD54, Flk1, and VEGF. In our opinion, $CD34^+/CD90^+$ ASC sub-population is the best candidate for endothelial differentiation. There is good evidence, in fact, that this sub-population produces both anti-apoptotic and angiogenic factors, including vascular endothelial growth factor (*VEGF*), hematopoietic growth factor (*HGF*), and TGF-β.[28] Since VEGF is necessary to promote endothelial differentiation, we found, consistent with these molecular data, that $CD34^+/CD90^+$ cells differentiated into endothelial capillary-like structures. Other authors used an *in vivo* nude mouse model of hind limb ischemia and demonstrated that ASCs injection markedly improved limb perfusion, which was coherent with an increase in blood flow and capillary density in the limb[39,72] (Fig. 2).

Myogenic lineage

Smooth and skeletal muscle cells have generally been reproduced by differentiating the ASC in complete media with 5% horse serum, and glucocorticoid such as hydrocortisone and/or dexamethasone. When cells are cultured under these conditions, they appear to merge and form multi-nucleated myotubes, generating protein markers for myocytes. ASCs, cultured as mentioned earlier, express mRNAs encoding myogenic proteins, including the transcriptional regulators MyoD and myogenin as well as structural proteins such as myosin heavy chain.[73] The capacity of ASCs to differentiate into muscle was recently demonstrated both *in vitro* and *in vivo*.[74] Two mechanisms are fundamental for muscle regeneration from ASC, as a result of the *de novo* formation of muscle-specific cells from ASCs and/or the change in gene expression after direct fusion of ASCs with host cells (Fig. 2). The myogenic potential of ASCs may be harvested in the treatment of Duchenne muscular dystrophy (DMD). *In vivo* murine studies have demonstrated that dystrophin expression may be restored over the long term, and *in vitro* studies have shown that ASCs can fuse with DMD myoblasts to reestablish dystrophin expression. This symbolizes a promising challenge in the use of stem cell therapy for muscular degenerative diseases.

Cryopreservation of ASCs

The possibility of MSC cryopreservation may have a potential clinical use. This procedure is not normal routine clinical practice, but major improvements have been made in the cryopreservation of cells and tissues.[75] However, hematopoietic stem cells have been largely cryopreserved and subsequently employed with success for transplantation.[76] It is fundamental to minimize destruction of the stem cells during the cooling and thawing processes. Therefore, optimization of cryopreservation protocols aimed at the maintenance of stem cell quality is a key factor in their banking. Dimethyl sulfoxide (DMSO) is commonly used as a cryopreservant for cells, but it is widely known to be toxic at room temperature. Trehalose is a nontoxic disaccharide of glucose that may stabilize and preserve cells and cellular structures during the freezing procedure. In particular, cryopreservation of adipose tissue has been studied extensively,[77] and some authors have demonstrated both *in vivo* and *in vitro* that trehalose adequately protects human fat grafts.[78,79] We based our previous study on these indications[80], and we observed the ability of ASCs to be cryopreserved using a solution of 4% DMSO and 6% trehalose plus 90% fetal bovine serum (FBS). This method of cryopreservation showed successful preservation of the ASCs after 1 year. According to De Rosa and colleagues, trehalose adequately protects proteins and membranes and preserves the latter even during dehydration. Due to its stabilizing effect, trehalose has recently been used in various biomedical,[81] cosmetic, and pharmaceutical applications[82], which also include solutions for vaccines and organs in surgical transplant.[83] A cryopreservation method using trehalose as a cryoprotective agent is recommended for a long-term preservation of ASCs compared with simple cryopreservation or with the method of DMSO cryopreservation alone. Trehalose is a white, odorless powder with a 45% relative sweetness of sucrose. Trehalose is a bisacetal, nonreducing homodisaccharide with two linked glucose units. Trehalose is an important bioprotectant thanks to the existence of a number of polymorphs. Furthermore, it is able to transit between crystalline form and another without losing its structural integrity. It may prevent the inactivation and aggregation of proteins at lower temperatures and may stabilize cell membrane by delaying the initial transformation from liquid crystal to gel state. Trehalose may also increase cytoplasmic viscosity and therefore may reduce the possibility of intracellular ice crystal formation that is often fatal to cells. Elucidations on the ''exceptional'' bioprotective properties of trehalose have been extensively and sufficiently reported by Jain and Roy,[84] which may be summarized as follows: (i) The vitrification theory that hypothesizes that trehalose forms a glassy matrix acting as a cocoon that physically shields the protein or even the cells from abiotic stress; (ii) the preferential exclusion theory that suggests that there is no direct interaction between trehalose and protein; and (iii) the water replacement theory that proposes that the substitution of water molecules by trehalose-forming hydrogen bonds maintains the 3D structure and stabilizes biomolecules. The effects of trehalose are most likely due to the combination of these theories.

hASCs Used for Adipose Tissue Engineering

D'Andrea *et al.*[85] performed, for the first time, a long-term culture of sorted hASCs in Dulbecco's modified Eagle's medium supplemented with 20% FBS for 4 months. These cells formed aggregates at confluence, resulting in an ECM

enriched with collagen fibers. Allowing these newly formed nodules to grow, they formed a layer of univacuolar adipocytes. Histological analyses of these structures showed that adipose cells surround a central area of fibrous connective tissue that is clearly positive for the connective/adipose markers that are type I collagen, fibronectin, adiponectin, and PPARγ as well as for endothelial markers such as VEGF, CD31, CD44, and CD54. This intriguing model of synergic differentiation is of great importance in clinical practice, since the co-differentiation of blood vessels is necessary to build new healthy tissue in regenerative therapies.

The importance of ECM is well acquainted not only for its chemical properties but also for its mechanical and physical properties that influence cell behavior. Investigators aim at combining what they know about the tissue-specific characteristics of ECM and how it alters with age and diseases with materials science to discover new constructs to be incorporated into the host system to restore tissue function. Tissue-specific scaffolds and signaling systems are fundamental to make stem cells differentiate into the required cells and for effective use in constructing 3D tissues. Adipose tissue engineering may be a promising and adequate solution to these problems with the use of hASCs, implanted on 3D biomaterials: a tissue-engineered substitute that promotes large-volume regeneration of the subcutaneous adipose tissue layer.

In summary, when hASCs are seeded on scaffolds for adipose tissue generation, various crucial factors need to be considered: the chemical composition, the mechanical stability, and the 3D architecture of the carrier. *In vivo* construct efficiency is likely to depend on the efficacy of the inoculation *in vitro* of hASCs into the scaffold and on the structure and chemical type of polymer utilized. In fact, when hASCs differentiate into adipocytes, they gain a significant volume; hence, a structure that is not growth impeding and permits stem cell differentiation is necessary. Moreover, the biomaterial should maintain the stability and the general morphology until replaced by adipose tissue, as well as allowing biodegradation after growth achievement. Besides, as previously mentioned, the matrix of the ideal carrier should favor the interplay between angiogenesis and adipogenesis, minimizing cellular ischemia.

Micronized acellular dermal matrix (micronized Alloderm)[86]

Micronized acellular derma matrix is a material that may be injected. It is versatile due to its intermediate-length implant and many applications. Studies have shown that Alloderm is an ideal scaffold for skin and oral mucosa in tissue engineering. Moreover, Alloderm is able to coordinate the arrangement of seeded keratinocytes and cultured keratinocyte sheets into a 3D epidermal structure with multiple layers and to recreate the original rete ridges at the interface of the dermis.[87] Yoo and Lim have experienced a hASC-Alloderm complex that, after 2 months *in vivo*, presented many signet-ring cells, large capillaries, and differentiated adipocytes that contained many lipid droplets. This injectable-engineered adipose tissue can be useful to augment soft tissue. As for other injectable scaffolds, the area of soft tissue that can be increased is limited; clinical trials are needed to evaluate how much lost tissue can be regenerated.

Moreover, similar scaffold materials, such as acellular dermis as well as small intestinal submucosa, amniotic membrane tissue, cadaveric fascia, and the bladder acellular matrix graft, on implantation, evoke a host-tissue response that originates from angiogenesis and aids tissue deposition, leading to restoration and repair.

Silk fibroin 3D scaffold

Silk-based biomaterial may prove beneficial in adipose tissue engineering applications due to its absence of bio burdens, low immunogenicity, slow degradation rate, plasticity during processing, and remarkable mechanical properties; they have been shown to offer exceptional benefit in producing functional tissue replacement for mesenchymal tissues, such as ligament, cartilage, and bone.[88] Mauney utilized ASCs seeded on a 3D porous silk-based fibroin scaffold for an *in vivo* study. After 4 weeks, a noteworthy increase in the frequency of lipid accumulating cells was noted in the silk fibroin scaffold, demonstrating that silk-based biomaterials also endorse *in vivo* adipogenesis. Altman *et al.* also reported that hASCs seeded on a silk fibroin-chitosan scaffold improved wound healing and displayed differentiation into epithelial, endothelial, and fibrovascular components of restored tissue.[89]

In conclusion, silk may be considered beneficial for tissue engineering applications thanks to its low immunogenicity, slow degradation, and strong mechanical characteristics. Notwithstanding these favorable features, the lack of potential in long-term *in vivo* soft tissues engineering applications may be a current limitation.

Polyglycolic acid and poly(lactic-co-glycolic acid)

Cho *et al.*[90] engineered a dome-shaped mechanical support, constructed by reinforcing poly(glycolic acid) fiber-based matrices with poly(L-lactic acid), seeded with hASCs, and implanted into subcutaneous pockets of athymic mice. Regeneration of adipose tissue in the construct was observed at 6 weeks after implantation. Kang *et al.*[91] used hASCs seeded on polyglycolic acid (PGA) and cultured in adipogenic medium for 2 weeks before their implantation in immune-deficient mice. The implants had developed new adipose tissue and had maintained their natural dimensions and shape, and novel blood vessels were observed in the newly formed adipose tissue. Injectable poly(lactic-*co*-glycolic acid) (PLGA) spheres with hASCs were employed as a noninvasive soft-tissue filler in a previous study.[92] PLGA has aroused considerable interest in clinical applications considering its biocompatibility; its biodegradation rate; its approval for clinical use in humans by the U.S. Food and Drug Administration (FDA); and its ability to modify surface properties to provide better interaction cells/biomaterial. PLGA is a linear copolymer that may have different preparation ratios between lactic acid (LA) and glycolic acid (GA). Different forms of PLGA may be acquired based on the ratio of the constituent monomers used for polymerization. PLGA may be dissolved in various solvents, such as chlorinated solvents, acetone, or ethyl acetate. Moreover, it may be processed into any size and shape, and may be encapsulated in different-sized biomolecules. The physical properties of PLGA seem to rely on various factors, including the initial molecular weight of the monomers, the LA:GA ratio, the exposure

time to water, and the storage temperature. These key characteristics enable an accessible use of PLGA biomaterials in clinical practice without provoking adverse effects in patients.

Similarly, PGA has been widely used in tissue engineering because of its chemical and mechanical properties. PGA has hydrolytically labile ester bonds and is degraded by nonenzymatic hydrolysis. The degradation is nontoxic and is eliminated from the body as carbon dioxide and water. Since this polymer is thermoplastic, it is readily manufactured according to the different tissue specifications. However, PGA may provoke inflammation in the transplantation site due to the lymphocyte homing.

Collagen sponge

Collagen, one of the most common biodegradable carriers, is commonly used in tissue engineering due to the porous matrix property, which supports cellular growth and new matrix synthesis.[93] Primarily, collagen biomaterials presented nonhomogeneous structures with differences in pore size[94] that did not lead to cellular implant and growth; progress in the manufacture of collagen matrix has resulted in a regular architecture of the scaffold.[95] *In vivo* the construct collagen/hASCs displays a significant neovascularization and adipocyte differentiation.[96,97]

Collagenous microbeads are a favorable scaffold for ASC, eliciting *ex vivo* proliferation and differentiation in small injectable particles.[98] Studies have shown that adipose tissues engineered with hASCs and type I collagen scaffolds may be used *in vivo* as a substitute to the damaged tissue. In fact, a recent study has provided evidence that hASCs can attach, grow, and proliferate on the collagen I scaffold because they are compatible at cellular level and considered an adequate vehicle for adipose tissue engineering and for production of loose connective tissue.[99]

There has been widespread use of collagen for soft tissue engineering. It offers, as a native tissue, biocompatibility, biodegradability, and weak antigenicity and is accessible in different forms as in 3D gels, fibers, scaffolds or sponges, and microbeads. Studies have demonstrated that type I collagen is highly compatible with living organisms and is largely used as a scaffold for adipose tissue engineering considering that it is the main fiber of the extra-cellular matrix in connective tissues.

Fibrin gel

Fibrin in the blood has a fundamental role in hemostatis with access via autologous plasma found in glue form or as engineered microbeads. This natural biomaterial is a versatile polymer and may be effective in wound healing.[100] For this reason, fibrin gel has strong neovascularization and tissue regeneration properties and it is used in a number of applications in tissue engineering.[101,102] hASCs embedded in a 3D fibrin gel have the ability to modulate vascularization of a healing wound.[103] However, the adipogenic characteristics of fibrin have not yet been tested.

Hyaluronic acid scaffold

Hyaluronic acid (HA) is a novel material used in tissue engineering; it is available in the extra-cellular matrix of several tissues, and it is assumed to support differentiation stimulating the tissue repair.[104,105] HA pore varied from 50 to 340 μm. Evidence has shown that sponge type prevails over other compositions with regards to weight, homogeneous distribution, and differentiated adipose tissue rate. In 3 to 8 weeks,[106] the construct HA/hASCs led to the formation of adipose tissue, fundamental for its resistance to swelling and for the diameter of the interconnected pores (120 μm). Hemmrich *et al.* in *in vivo* trials evaluated new sponge architecture with pores of 400 μm diameter that yielded more satisfying results in terms of vascularization, cell diffusion, and proliferation than collagen sponge with a 65 and 100 μm pore size. *In vivo* studies have demonstrated the presence of an extensive formation of newly formed vessels in all sponge depths after 12 weeks.[107] However, the new tissue was not totally formed by mature adipocytes. A possible explanation is that the 400 μm pore size is too big to give the key stimuli of contact inhibition that let the cells go through differentiation.[108] A probable solution is to synthesize a structure with a variable pore size between 100 and 400 μm and to increase the number of seeded ASCs.

Although hyaluronan 3D scaffolds may be considered an adequate 3D carrier for the culture and *in vitro* differentiation of human adipocyte precursor cells, so far they have not been particularly successful in adipose outcomes.

Adipose tissue as a scaffold: cell-assisted lipotransfer

In 2006, Matsumoto *et al.*[109] developed a new method for adipose tissue transplantation, called cell-assisted lipotransfer (CAL). In CAL, 50% of the aspirated fat volume is prepared to be isolated in the SVF-containing hASCs. During the isolation process, the remaining amount of the aspirated fat will be used for grafting. Freshly isolated SVFs, containing hASCs, blood cells (WBCs and RBCs), pericytes, vascular endothelial cells, and other cells, adhere to the aspirated fat that serves as a living scaffold before transplantation. The authors transplanted fat portion without hASCs (non-CAL) or CAL portion in SCID mice. Four weeks later, they compared the amount of fat tissue in CAL and non-CAL samples and noticed that CAL fat had a higher survival rate than non-CAL fat and microvasculature was more evidently seen in CAL fat. Two years later, Yoshimura *et al.*[110] applied this novel strategy for facial lipoatrophy and for breast augmentation with excellent results. In the CAL strategy, autologous hASCs are adopted to promote angiogenesis, as well as to ameliorate the survival rate of grafts, and to diminish postoperative atrophy.

hASCs Used to Build Other Tissues Different from Adipose Tissue

Using this attractive cell population, recent studies have explored the safety and efficacy of implanted/administrated hASCs in various animal models. Furthermore, clinical trials using hASCs have been initiated in some medical subspecialties.

Tissue engineering for organ reconstruction is extensively required in injuries and medical conditions but is mostly considered beneficial in the treatment of deep burns in response to its use of living substitutes to permanently replace the epithelia. The technique of skin tissue engineering has been the subject of various studies.[111,112]

Bi-layered skin substitutes, including the epidermis and the dermis, have become fundamental in developing the dermal matrices over recent years together with the autologous fibroblasts that aid in the repair mechanism of burn wound healing.[113] Trottier *et al.*[114] defined the mechanism of producing novel human skin substitutes starting from keratinocytes and hASCs *in vitro* without the support of exogenous or synthetic scaffolds. These substitutes showed similar characteristics on a histological and immunohistological level as the dermal fibroblast substitutes.

Recent progress in bone tissue engineering has focused on developing biological substitutes to regenerate, preserve, or ameliorate tissue function. One of the primary targets of clinical therapeutic strategies is the regeneration of bone to repair large bone defects caused by traumatic events, infection, tumor enucleation, and skeletal deformities. Autologous grafts represent the ideal graft bone substitutes. An alternative approach involves the use of allogenic bone grafts taken from corpses or living donors. The limited success of auto and allografts has stimulated the investigation of a wide variety of biomaterials for scaffold use in *in vitro* tissue engineering. Many types of materials have been used to confirm the availability of ASCs for bone tissue engineering, including ceramics,[115] titanium scaffold,[116] natural and synthetic polymers[117,118] with variable porosity, roughness, and methods of fabrication. Differences in currently applied techniques make studies complicated to compare and the lack of guidelines to control safety in the use of the different materials involved in bone scaffolds may be a matter of concern. Prospective randomized clinical trials are needed to demonstrate clinical outcomes of the ASCs therapies.

Cartilage tissue engineering has been increasingly explored in the past few years. The complexity and the specificity of cartilage reside in its aneural, avascular, and alymphatic nature. In this context, cartilage regeneration represents one of the most difficult dares in tissue engineering and clinical applications. Novel scaffolds, which facilitate the differentiation of ASCs into cartilaginous phenotype, play an important role. A broad range of natural polymers have been studied as scaffolds[119,120] together with synthetic materials such as PLGA and others,[121,122] as well as chitosan-HA.[123] In this scenario, it is important to mimic the cartilage tissue environment (by tridimensional application) and to use prochondrogenic factors in the design of biomaterials such as HA and chondroitin sulfate.

ASCs have been recognized as a promising tool to address the cure of liver cirrhosis. Interestingly, Ruiz *et al.*,[124] confirming and extending the previous report,[125] documented that hASCs were able to develop the biochemical features of mature hepatocytes after induction using a successful combination of HGF and Oncostatin M induction *in vitro*. The *in vivo* study demonstrates that hASCs and ASC-derived hepatic cells are closely embodied in the liver and form large cords of parenchymal tissue. These data implicate the permissive role of the adult liver environment in hASC hepatogenesis.

Acute and chronic ischemic heart diseases are among the main causes of mortality worldwide. Conventional management generally does not replace cardiomyocyte mass loss or myocardial fibrotic tissue. hASCs extemporaneously differentiate into cardiomyocytes *in vitro*, express the cardiac-specific markers troponin-I and myosin light chain 2,[126,127] and spontaneously and rhythmically contract.[37] However, the *in vivo* differentiation of hASCs into cardiomyocytes is still considered a controversial issue.

The myogenic properties of hASCs can be exploited in the treatment of DMD, an inherited genetic disorder progressively leading to skeletal muscle degeneration. *In vivo* murine studies were carried out *in vivo* to show that hASCs implanted into dystrophin-deficient immunocompetent mice restored dystrophin expression in the muscle injection site and in the surrounding muscles over the long term.[128] The reconstruction of skeletal muscle tissue *in vitro* has been widely endeavored, and the idea of engineering new fibres has become an attractive option since the introduction of ablation procedures by surgery or from major traumas.[129] Besides, numerous scaffold materials have been studied for auto-transplantation for both *in vitro* and/or *in vivo* growth and differentiation. For example, HA scaffolds have been employed as a cell-carrier material and play an important role in cellular behavior. In a recent study, the possible use of a modified crosslinked HA scaffold on different subpopulation of hASCs has been evaluated. The authors pinpointed a subpopulation of CD34-positive cells also expressing NG2, a perycite marker, according to Traktuev *et al.*[42] The $CD34^+/NG2^+$ population, seeded onto this new scaffold, leads to differentiation into skeletal muscle tissue. The acquired muscle tissue was made up of an organization of myofibers showing peripherally located nuclei of human origin, confirmed as positive (for I Class HLA and MyoD expression.[130]

Conclusion

The potential use of ASCs in plastic surgery is unlimited and a wide range of treatments and reconstructions are necessary to be used in different fields ranging from oncology to aesthetics, to adequately address individual patient needs. The use of adipose tissue-derived stem cells as a therapeutic tool has grown substantially in the past decade and has stimulated the growth of a new research field and industry worldwide. Adipose-derived cells may be used in a wide range of clinical disorders depending on the target tissue or on the underlying condition (immunological or ischemic). Nevertheless, there are still some unclear aspects in the basic knowledge of ASCs biology and the clinical applications[29] regarding the identity of adipose tissue-derived cell populations and their use in clinical practice. The IFATS and International Society for Cellular Therapy (ISCT) provide initial guidance to academia and industries regarding the properties of adipose tissue-derived cells. The guidelines for the characterization of hASCs regard the viability, standard immunophenotype, proliferation, and differentiation. These features facilitate the development of further endpoints and accelerate safe and effective delivery of hASC-based tools to the medical community and the patients. ASCs have prominent and strong involvements in tissue regeneration due to their high cell yield in adipose tissue, their ability to differentiate into multiple lineages, to secrete various cytokines and thanks to their immunomodulatory

effects. A wide number of clinical trials using ASCs have already been performed, and many of them are ongoing. ASCs are a promising cell source for regenerative medicine, and further research is necessitated to guarantee the safety of ASCs and the effectiveness of tissue engineering using ASCs. For this reason, it is important to define success rate of the engineered tissue before application; determine the biomechanical properties of the engineered tissue; develop guidelines to establish the biomechanical requirements of the tissue to be replaced; design and characterize the biomechanical and biophysical properties of the scaffold to be used; specify the microenvironment of the implant construct; and identify the potential risks for oncological patients.

These days, it is possible to employ stem cells acquired from adipose tissue or bone marrow in various ways. For example, they can be exploited for culture and expansion in adequate cell factories for tissue processing and generation of cells for therapeutic purposes. The adipose-derived stem cell field is at a turning point as it moves from basic science to clinical application. Solid research in the basic science and biology behind stem cells has been performed, but a gap still exists between basic knowledge and clinical application. In the past decade, several skills have been obtained regarding isolation, morphological characteristics, molecular biology, and the differentiation potentiality of *in vitro* stem cells.

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Disclosure Statement

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