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Review Article

Adipose Tissue Regeneration: A State of the Art

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Adipose tissue pathologies and defects have always represented a reconstructive challenge for plastic surgeons. In more recent years, several allogenic and alloplastic materials have been developed and used as fillers for soft tissue defects. However, their clinical use has been limited by further documented complications, such as foreign-body reactions potentially affecting function, degradation over time, and the risk for immunogenicity. Tissue-engineering strategies are thus being investigated to develop methods for generating adipose tissue. This paper will discuss the current state of the art in adipose tissue engineering techniques, exploring the biomaterials used, stem cells application, culture strategies, and current regulatory framework that are in use are here described and discussed.

1. Introduction

Adipose tissue pathologies and defects have always represented a reconstructive challenge for plastic surgeons. Contour defects resulting from resection of tumors, as well as from trauma and congenital abnormalities not only affect patients cosmetically, but also may impair function, making adipose tissue restoration a strong clinical need [1]. Surgical strategies for tissue loss replacement initially laid on the historical maxim "replace tissue with like-tissue": fatty tissue has been transplanted since 1893, but literature has always shown controversial results in the degree of lasting of corrections, due to fat reabsorption [2]. In more recent years, several allogenic and alloplastic materials have been developed and used as fillers for soft tissue defects [3]. However, their clinical use has been limited by further documented complications, such as foreign-body reactions potentially affecting function [4], degradation over time [3], and the risk for immunogenicity [5]. Tissue-engineering strategies are thus being investigated to develop methods for generating adipose tissue.

The emerging field of adipose tissue engineering aims at developing biologic substitutes that promote regeneration and restore function, through the application of the principles and methods of engineering and the life science [6]. This revolutionary approach leads to the regeneration of healthy tissues or organs for patients, thus eliminating the need for tissue or organ transplants and mechanical devices [7]. Specifically, cells are harvested from a patient and amplified in laboratory. Afterwards, they can be seeded onto a scaffold that will support cell growth and proliferation. The cell-covered scaffold may then be implanted into the patient at the needed site. After guiding tissue regeneration, the scaffold will then degrade, leaving a newly formed tissue [8]. The development of adipose tissue-engineering strategies requires investigation of all key aspects of the tissue engineering process, including the selection of cell source,

Table 1: Currently available biomaterials for adipose tissue regeneration.

References	Biomaterial	Origin
[9–12]	Poly(lactic acid)	Synthetic
[9–13]	Poly(glycolic acid)	Synthetic
[14]	Poly(ethylene glycol)	Synthetic
[15–17]	Poly(lactic-co-glycolic acid)	Synthetic
[11]	Polyethylene terephthalate	Synthetic
[11]	Polypropylene	Synthetic
[18–22]	Collagen	Natural
[23, 24]	Silk	Natural
[25, 26]	Adipose-derived ECM	Natural
[27]	Placental decellularized matrix	
[28]	PDM cross-linked hyaluronan	Natural
[29]	Omenta	
[30]	Fibrin	Natural
[19, 20]	Gelatin	Natural
[31, 32]	Hyaluronan	Natural
[33, 34]	Matrigel	Natural

scaffold biomaterial, and microenvironment to provide the appropriate cues and signals for cell growth and adipose tissue formation [35]. (Finally, the successful *in vitro* production of human adipose substitutes featuring an increased surface area (>30 cm^2) is described, reinforcing the notion that customized autologous reconstructed adipose tissues could be produced in the future to repair a wide range of soft-tissue defects.) This paper will discuss the current state of the art in adipose tissue engineering, exploring the biomaterials, cell sources, and culture strategies that are currently in use.

2. Biomaterials for Adipose Tissue Regeneration

The challenge represented by reconstruction of functional adipose tissue is due to its high-dependence on a patent microvasculature and a preserved extracellular matrix (ECM) in order to ensure viability of the transplanted or regenerated tissue [36]. The role played by the ECM is well known to be crucial not only for its chemical, but also mechanical and physical properties in influencing cell behaviour [37]. By combining the knowledge of tissue-specific ECM properties and its alteration with age and diseases [38] with materials science, researchers seek to create novel constructs that will fully integrate into the host system and restore function.

Many materials have been explored for adipose tissue engineering, both synthetic and natural (Table 1). Advantages and disadvantages with respect to material biocompatibility, mechanical and chemical properties, and degradability have been reported. Ideally, the complications associated with the current treatment strategies would be progressively eliminated. We will describe currently available biomaterials for adipose tissue regeneration.

2.1. Synthetic Polymers. Synthetic polymers have been widely utilized in adipose tissue engineering. Significant advantages include the ability to specifically tailor a synthetic polymer with respect to its mechanical properties, chemical properties, and degradability [9]. For soft tissue applications, polymers as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ethylene glycol) (PEG), and the copolymer poly(lactic-co-glycolic acid) (PLGA) have been extensively used.

PLA and PGA chemical-physical properties (controlled degradability by alteration of molecule weight [9]) have been utilized for both *in vitro* and *in vivo* studies as 3D scaffolds or grafts for adipose tissue engineering, showing potential in supporting tissue regeneration [10–12]. PGA meshes proregenerative properties have been elucidated by Weiser et al. [13]. The long-term availability of PGA meshes *in vivo* (12 weeks) supported adipogenesis and vascularization, which were evident at 24 weeks [13]. PLA scaffolds were investigated as well *in vivo* for adipogenesis support, however not providing detectable data at the end of the 4-week study [11]. The copolymer PLGA has been utilized for engineered constructs, featuring human-rat adipocytes and additional growth factors (bFGF) on a 3D scaffold, which have been shown to induce neovascularization *in vivo* [15, 16]. Moreover, PLGA is a useful biomaterial for growth factors embedded engineered microspheres, which promote a time- and space-controlled release of growth factors (bFGF) to promote proliferation and differentiation in the matrix [17]. While it appears that PEG hydrogels can be designed to permit adipogenesis in response to soluble signals *in vitro* [14], conditions have not been identified that result in a material that specifically improve adipogenesis. It is likely that additional, adipose-specific signals need to be incorporated into these synthetic hydrogels.

These strategies related to the incorporation of growth factor into the scaffold in order to promote adipose tissue regeneration have been used by other researchers as well as Zhu et al. [39] by the development of multiple growth factors incorporated into nanosphere-coated microspheres or by Song et al. that use VEGF in adipose tissue regeneration [40]. In the end, Garten et al. [41] report several studies of insulin-like growth factors in adipogenesis. Adipose tissue has been recognized as a major target of growth hormone (GH) action. GH was shown to inhibit adipocyte differentiation but stimulated preadipocyte proliferation *in vitro*. GH acts directly via its receptor or via upregulating insulin-like growth factor (IGF)-I, which is a critical mediator of preadipocyte proliferation, differentiation, and survival. Results from clinical studies on GH treatment in patients with GH deficiency or GH insensitivity syndrome can be used to dissect GH and IGF as well as IGF-binding protein (IGFBP) actions *in vivo*. depots as well as the recently emerging role for adipose tissue in the regulation of glucose homeostasis [41].

Other synthetic materials have also been explored for adipose tissue engineering applications, some showing promise for potential soft tissue replacement. These include 3D scaffolds and meshes composed of polyethylene terephthalate and polypropylene [11], and various silicone tubing/housing structures for adipose tissue engineering support [42].

2.2. Natural Polymers. As natural polymers are part of the native ECM, their use for tissue engineering applications poses advantages with respect to biocompatibility, mechanical, and biological properties, which tend to match those found *in vivo*. Natural polymers that have been explored nowadays include collagen, silk fibroin, adipose-derived ECM, decellularized human placenta, fibrin, gelatin, hyaluronan, and matrigel.

Collagen has been widely used for soft tissue engineering: as a native tissue, it provides biodegradability, biocompatibility, and weak antigenicity [18]. Collagen has been utilized in many forms, including 3D gels, fibers, scaffolds or sponges, and microbeads. There are a number of studies demonstrating the ability of 3D collagen sponges to support adipogenesis from various cell sources [19, 20], as well as promote development of new adipose tissue *in vivo* after 12 weeks [21]. Some recent developments include the addition of short collagen fibers within collagen gels to reduce contraction, which increased cell viability and lipid accumulation *in vitro* [22]. Collagen hydrogels containing FGF-2 encapsulated within gelatin microspheres have been shown to facilitate the development of vascularized adipose tissue *in vivo* even without the addition of a cell source [18].

Silk is a biomaterial that shows favourable characteristics for various tissue engineering applications, such as low immunogenicity, slow degradation, and strong mechanical properties [23]. The ability to tailor silk fibroin as a biomaterial with respect to its biodegradability, mechanical strength, and ability to undergo surface modifications provides strong rationale for the use of silk as a scaffold for soft tissue regeneration [43–45]. At the current moment, interesting results on the ability to engineer vascularized adipose tissue have been achieved *in vitro* by a coculture of adypocytes with endothelial cells in an aqueous-based silk fibroin scaffold [24]. However, the current limitation to its acknowledged potential is the lack of long-term (more than 6 months) *in vivo* soft tissue engineering applications.

In an attempt to create an entirely autologous tissue-engineered adipose substitute, researchers extracted human stromal cells from either lipoaspirated or resected fat and assessed their capacity to produce a three-dimensional adipose tissue using an adapted "self-assembly" culture methodology. When viewed by scanning electron microscopy, the appearance of these reconstructed adipose tissues was strikingly similar to subcutaneous fat. Furthermore, these substitutes secreted adipokines and mediated beta-adrenergic receptor-stimulated lipolysis, hence reproducing known major biological functions of white adipose tissue [25]. On a functional level, the reconstructed adipose tissues expressed adipocyte-related transcripts and secreted adipokines typical of adipose tissue, such as leptin. The adipose tissue engineering research has focused on the investigation of naturally derived placental decellular matrix (PDM) and PDM cross-linked hyaluronan (XLHA) scaffolds for soft tissue augmentation [26, 27]. Scaffolds seeded with primary human adipose-derived stem cells (ASC) were investigated in a subcutaneous athymic mouse model. At 3 and 8 weeks, both scaffolds macroscopically maintained their three-dimensional volume and supported mature adipocyte populations *in vivo*. There was evidence of implant integration and a host contribution to the adipogenic response. The results suggested that incorporating the XLHA had a positive effect in terms of angiogenesis and adipogenesis [28]. Overall, the PDM and PDM with XLHA scaffolds showed great promise for adipose tissue regeneration. However, PDM engineering techniques will probably have to be further improved for extensive clinical use, as isolation and decellularization procedures still require a minimum of 18 days. Recently, a novel source of decellularized scaffold has been proposed for further recellularization by autologous cells isolated from lipoaspirate samples [29]. Adult rat and human omenta were decellularized maintaining a complex 3D collagenic structure, preserving the architecture of the tissue. Preliminary analysis suggests the possibility of recolonization *in vitro* of the scaffold by adipose-derived stem cells. Avoiding rejection, the scaffold could be particularly suitable for reconstructive applications *in vivo*.

Potent neovascularization and tissue regeneration properties of fibrin have led to its exploration in a number of applications in tissue engineering. Fibrin gels can be used as a carrier for injected adipocytes that induce adipose tissue regeneration when implanted in rodent models [30]. However, the adipogenic properties of the fibrin has not been tested yet in a 3D porous scaffold.

Gelatin, as a natural derivative of collagen, has found several applications in bioengineering applied to tissue regeneration. Gelatin does not exhibit antigenicity, and it is one of the most affordable proteins to use. Actually, investigation to evaluate *in situ* adipose tissue regeneration took advantage of gelatin microspheres prepared for the controlled release of bFGF, either in a collagen sponge scaffold [19] or on a basement membrane extract (Matrigel) [20]. Histologically evaluated adipogenesis was enhanced with time after implantation up to 4 weeks in both approaches.

Hyaluronic acid-based materials have been recently used for engineering of several different tissues (e.g., skin [30], cartilage [46], and bone [47]). Experiments also demonstrated that human preadipocytes can be attached to hyaluronan-based scaffolds. Implantation of such bioconstructs into nude mice resulted in local differentiation of preadipocytes suggesting that this approach is feasible at least in an animal model [31]. Among the different scaffold types available, the highest cell attachment rate has been observed for the HYAFF scaffold with 100% esterification and a mean pore size of 400 mm (HYAFF 11lp) [32]. Although hyaluronan 3D scaffolds appear to be a suitable three-dimensional carrier for the culture and *in vitro* differentiation of human adipocyte precursor cells, they have not been widely successful for adipose outcomes yet.

Matrigel is a commercially available product extracted from a spontaneously occurring mouse sarcoma [33]. It consists primarily of laminin-111, collagen IV, and entactin at ratios similar to those found *in vivo*. Interest in Matrigel for adipose tissue engineering derives from studies showing that the gels induce neovascularization and adipose formation in rodents when supplemented with more than 1 ng/mL of FGF-2 and injected subcutaneously [33]. Matrigel has also been shown to improve the longevity and volume

maintenance of adipose grafts when mixed with adipocytes [48]. (Although Matrigel has shown to sustain extensive adipose tissue formation, concerns about its tumoral xenogenic nature posed limitations to the possible applications.) Moreover, Matrigel has not been demonstrated to be adipogenic in the absence of growth factors, suggesting a more conductive rather than inductive effect. A potential alternative has been developed from skeletal muscle extracts (Myogel) supporting preadipocyte differentiation *in vitro* and vascularized adipose formation *in vivo*. Interestingly, the results *in vivo* occurred without the addition of exogenous growth factors [34]. However, it remains unclear why muscle-based materials would provide an improved environment for adipogenesis over materials from other tissue sources.

3. Adipose-Derived Stem Cells for Adipose Tissue Engineering

Critical to the cell-based therapy success is the viability of cells, that is based on the full integration of cells and on the blood, oxygen, and nutrients supply [49]. Because MSC are multipotent cells that are easily isolated, easily cultured, and readily expanded in the laboratory setting, they became an attractive cell source for use in several clinical applications [50, 51]. Interestingly, adipose tissue seems to be a particularly good source of stem cells and it contains more multipotent cells per cc than bone marrow: one gram of adipose tissue yields $\sim 5 \times 10^3$ stem cells, which is 100-fold higher than the number of mesenchymal stem cells in one gram of bone marrow [52, 53]. Adipose tissue, harvested through aspiration, can be processed manually or automatically to remove the mature fat cells and obtain a heterogeneous mixture of cells, where 5% is represented by stem cells [54]. These cells are called adipose-tissue-derived stem cells (ADSCs) and they are routinely isolated from the stromal vascular fraction of homogenized adipose tissue. Similar to MSCs, ADSC have been shown to reside in a perivascular location, and increasing evidence shows that both MSC and ADSC may in fact be vascular stem cells. Locally, these cells differentiate into smooth muscle and endothelial cells that are assembled into newly formed blood vessels during angiogenesis and neovasculogenesis. Additionally, MSC or ADSC can also differentiate into tissue cells such as adipocytes in the adipose tissue. Systematically, MSCs or ADSCs are recruited to injury sites where they participate in the repair/regeneration of the injured tissue [55–57].

Today, adipose tissue aspiration is a commonly performed surgical procedure, and it is relatively easy to harvest a large volume of tissue, obtaining an abundance of isolated stem and therapeutically active cells without the requirement of cell expansion in tissue culture facilities. This approach may mark the beginning of a new era in regenerative medicine and clinical reconstruction.

ADSC have potential applications for a wide range of clinical disorders. To further advance in the clinical utility of this cell therapy, a greater understanding of the mechanisms of interactions among ADSC, secreted growth factors, and biomaterials on tissue regeneration is needed.

3.1. Adipose-Derived Stem Cells. For many years, bone-marrow-derived stem cells (BMSCs) were the primary source of stem cells for tissue engineering applications [58–60]. Recent studies have shown that subcutaneous adipose tissue provides a clear advantage over other stem cell sources due to the ease with which adipose tissue can be accessed (under local anesthesia and with minimum of patient discomfort) as well as to the ease of isolating stem cells from the harvested tissue [61, 62]. Moreover, stem cell frequency is significantly higher in adipose tissue than in bone marrow and the maintenance of the proliferating ability in culture seems to be superior in ADSC compared with BMSC [63].

Initial enzymatic digestion of harvested adipose tissue yields a mixture of stromal and vascular cells referred to as stromal-vascular fraction (SVF) [64]. SVF provides a rich source of pluripotent ADSC [61, 62], which were first identified by Zuk and named processed lipoaspirate (PLA) cells [52, 65, 66].

There is no consensus when it comes to the nomenclature used to describe these cells, variously termed preadipocytes, stromal cells, PLA cells, multipotent adipose-derived stem cells, or ADSC. However, at a consensus conference of the International Fat Applied Technology Society, the term "adipose-derived stem cells" (ADSCs) was recommended for consistency between research groups [67].

Besides nomenclature problems, the real critical point concerning ADSC is the absence of a consensus statement on minimal acceptance criteria, based on cell viability and/or proliferation rates, immunophenotype, and differentiation potential.

Morphologically, ADSC are fibroblast-like cells and preserve their shape after *in vitro* expansion [65, 68, 69]. Average doubling time of tissue cultured ADSC is between 4 to 5 days [70]. They are mesenchimal stem cells similar to BMSC. According to the literature, ADSC expression profile is very consistent and shares over 90% of the mesenchymal stem cell markers: CD9, CD10, CD13, CD29, CD44, CD54, CD55, CD71, CD73, CD90, CD105, CD146, CD166, and STRO-1. They are negative for the hematopoietic lineage markers: c-kit, HLA-DR, CD4, CD11b, CD14, CD16, CD45, CD56, CD62E, CD79, CD104 CD117, and CD106 and for the endothelial markers: CD31, CD144, and von Willebrand factor (the adipose-tissue-derived stromal cell expression of surface markers and genes is summarized according to data derived from the literature [66, 71]). *In vitro* culturing leads to changes in ADSC immunophenotype. The expression of CD34, MHC class I and II molecules, CD80, CD86, CD45, CD11a, CD14, CD117, HLA-DR, CDKN1B, INS, ITGA5, NOG, UTF1, WNT6, and WNT8A often decreases with culturing, while the expression of CD9, CD13, CD29, CD44, CD63, CD73, CD90, CD105, CD166, ACTG2, ACVR1, BMPR2, CTNNB1, CCNE1, CDH1, COL6A2, HSPA9, IL6, ITGA8, ITGB1, ITGB5, MDM2, PTEN, PUM2, SNAI2, TGFBR1, and VEGF-A tends to increase [72]. This results in a more homogeneous cell population with extended culturing [73]. The problem of the change of markers during *in vitro* expansion cannot be satisfyingly solved at present and more detailed molecular data are necessary before a clear knowledge of the global and specific gene

and protein expression profile of ADSC—prerequisite for a highly effective cell therapy—can be achieved.

Apart from the phenotypic characterization, other minimal criteria have been proposed to define MSC by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [74]. These are plastic adherence ability, tripotential mesodermal differentiation potency into osteoblasts, chondrocytes, and adipocytes and immunomodulatory capability [63]. Several groups demonstrated ADSC potency showing differentiation toward osteogenic, chondrogenic, adipogenic, myogenic, hepatic, or neurogenic lineages *in vitro* [66, 71, 75].

Concerning the immune regulatory capability and anti-inflammatory effects [76], several ADSC characteristics may be involved: ADSCs have low immunogenicity due to low expression of human leukocyte antigen (HLA) class I and no expression of class II molecules, as demonstrated by flow cytometry. Their immunomodulatory effect limits both alloantigen and mitogen-induced lymphocyte responses. Expanded ADSC can elicit lymphocyte proliferation neither after coculturing nor after mitogen stimulation and do not provoke alloreactivity of incompatible lymphocytes [77]. Moreover, passaged ADSC might serve as the third-party cells to inhibit the two-way mixed lymphocyte reaction. This type of immunosuppressive effect is not cell-cell contact dependent and PGE2 might be the major soluble factor involved in the *in vitro* inhibition of allogeneic lymphocyte reaction [78].

The lack of HLA-DR expression and the above described immunosuppressive properties of ADSC make them suitable even for allogenic transplantation procedures but this requires further investigation [79].

3.2. ADSC Isolation and Culture Procedures. The simple surgical procedure, the easy and repeatable access to the subcutaneous adipose tissue, and the uncomplicated enzyme-based isolation procedures make adipose tissue a very attractive source of stem cells for researchers and clinicians [61, 80]. ADSC prepared from human lipoaspirate for different studies differ in purity and molecular phenotype, with many groups using different heterogeneous cell preparations. Besides the lack of standardization between research groups in defining what is meant by ADSC, one of the most significant issues limiting the interpretation of results and clinical progression of ADSC research is the lack of a consensus on the way of isolating these cells. There is a strong need for optimization of ADSC isolation and propagation procedures for subsequent clinical use.

The state-of-the-art stem cell isolation technique includes several common steps to process cells from adipose tissue: washing; enzymatic digestion/mechanical disruption; filtration and centrifugal separation for isolation of cells which can be used directly, cryopreserved or expanded for the generation of ADSC. Oesayrajsingh-Varma and coworkers found that the yield of stromal vascular cells was approximately $0.5–0.7 \times 10^6$ cells/g adipose tissue, with about 82% viability after the extraction procedure, which was later confirmed by independent groups [62]. Harvest techniques might reduce this yield, even if metabolic characteristics and fat cell viability seem not to differ when comparing standard liposuction with syringe aspiration [81].

Factors such as donor age, donor BMI [82], type (white or brown adipose tissue) and localization (subcutaneous or visceral adipose tissue) of the adipose tissue, type of surgical procedure, culturing conditions, exposure to plastic, plating density, and media formulations might influence both proliferation rate and differentiation capacity of ADSC. A detailed comparison of five different subcutaneous depots determined, for example, that ADSC isolated from the arm and thigh best maintained adipogenic potential as a function of advancing age [83]. Further studies in larger cohorts will be necessary before patient demographics can be used to predict the functionality and recovery of SVF cells and ADSC from donors as well as the relative utility of specific depot sites.

Differences were investigated also concerning the isolation procedure: different collagenase batches and centrifugation speeds can cause the isolation of different cell subsets. Several enzymes were compared with respect of yield of nucleated cells and precursor cells. However, results showed that interdonor variability is greater than differences between individual enzymes [84, 85]. Moreover, the enzymes used to disrupt lipoaspirate tissue might contain, in their crude form, contaminating amounts of endotoxin, other peptidases, and xenoproteins [86]. To overcome this problem, functional ADSC can be expanded directly from lipoaspirate fluids by a mechanical device without the need for collagenase digestion [87]. This allows for reduction of adipose cell cluster size, while eliminating oil and blood residues from the final cell product suspension. The reduced particle size of adipose tissue allows stem cells to creep out of the tissue and within 7 days they are ready for the first passage. The development of an efficient and reproducible mechanical-based tissue disruption process would remove the need for enzyme reagents and merits further investigation.

Other companies have developed self-contained lipoaspirate processing devices that collect, wash, digest, and separate cells without exposing them to the environment [88]. These devices are relevant when speed of preparation is the essence for optimal surgical practice. Coarse preparations from lipoaspirate, such as SVF, are acceptable, because they are quick to prepare and will still provide ADSC into the graft, even though they are also likely to contain other cells. Hence, for some surgical applications, purity of ADSC may be sacrificed in favor of surgical convenience while for others, *in vitro* expansion is mandatory to generate the cell quantities required to achieve therapeutic results, especially through systemic delivery. In order to obtain clinically relevant yields of ADSC, 3–5 weeks of culturing are necessary and result in a homogeneous population of ADSC, which depending on the culture conditions, can maintain their multilineage differentiation potential [73].

On the whole, standardization of ADSC isolation (mechanically and/or enzimatically) and expansion procedures would allow direct comparison of results across all research groups and a better definition of their clinical potential versus any of the other cell populations present in SVF.

Table 2: Adipogenic differentiation of ADSC induced by medium supplementation.

Reference	Medium	Serum	Supplementation
[89]	DMEM/F-12	3% FBS	0.25 mM isobutylmethylxanthine (IBMX), 1 μM dexamethasone, 1 μM bovine insulin, 33 μM biotin, 17 μM pantothenate, 5 μM rosiglitazone, and 100 units of penicillin, 100 μg of streptomycin, and 0.25 μg of fungizone
[90]	Alpha-MEM	20% FBS	0.5 mM IBMX, 0.5 μM dexamethasone, 50 μM indomethacin, 1% L-glutamine, and 1% P/S
[91]	DMEM-high glucose	10% FBS	0.5 mM IBMX, 1 μM dexamethasone, 10 μg/mL insulin, 200 μM indomethacin, and 1% antibiotic

It is believed that culturing ADSC in the form of multicellular aggregates (MAs) can improve cell-cell signaling and cell-matrix signaling. ADSC within the MAs maintain the capacity to adhere to tissue culture plastic, as well as to proliferate and differentiate along multiple lineages. MA methods are also capable of reducing the donor-to-donor variability that exists when ADSC are cultured in monolayer [92]. MAs provide ADSC with a 3-dimensional growth microenvironment more similar to that found *in vivo* and this enhances proliferation, differentiation, and angiogenesis [93].

3.3. Mechanisms of Action. Given their properties, it is unsurprising that ADSC are entering clinical trial for numerous applications. The potential benefits of ADSC are promising for regenerative medicine because they have several advantages: ability to continue proliferating after transplantation [94], to differentiate into endothelial cells, to induce neovascularization [95], and to release angiogenic growth factors [35, 96]. ADSC secrete high levels of epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), transforming growth factor-beta (TGF-β), insulin-like growth factor (IGF), and brain-derived neurotrophic factor (BDNF) [97–102]. They also secrete cytokines such as Flt-3 ligand, granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-11 (IL-11), interleukin-12 (IL-12), leukemia inhibitory factor (LIF), and tumor necrosis factor-alpha (TNF-α) [56, 100]. These angiogenic and antiapoptotic growth factors are secreted in bioactive levels by ADSC and their secretion significantly increases under hypoxic conditions [56, 102, 103].

The broad range of clinical applications for ADSC largely depends on their potential for differentiation and on their ability to migrate and to recruit endogenous stem cells from the niches. Freshly isolated SVF contains a mixture of cells, which not only include ADSC but also contains endothelial cells, smooth muscle cells, pericytes, fibroblasts, and circulating cell types such as leukocytes, hematopoietic stem cells, or endothelial progenitor cells [65]. Among these cell types of mesodermal origin, the differentiation process can be switched, for example, by overexpression of lineage-specific transcription factors. The processes of proliferation, allocation, and lineage-specific terminal differentiation are regulated by a complex interplay involving stem cell transcription factors (molecular rheostats), cell-specific transcription factors, and a wide variety of cellular kinases, growth factors, and receptors. ADSC capability to differentiate towards adipogenic lineages has implications for breast soft tissue reconstruction after tumor surgery for breast cancer, breast asymmetry, and soft tissue and subdermal defects after trauma, surgery, or burn injury. As the differentiation of ADSC into adipocytes is not in any doubt, progression of related clinical treatments and trials is further advanced than for other differentiation lineages. Table 2 shows a list of the protocols currently used for inducing adipogenic differentiation of ADSC. ADSC chondrogenic commitment is relevant for joint and disc defects repair and for plastic reconstruction of ear and nose defects. They can be used for skeletal regeneration of inherited and tumor- or trauma-induced bone defects, thanks to their osteogenic differentiation. Given their myogenic and cardiomyogenic properties, ADSCs are useful for muscle reconstruction after trauma and surgery, dystrophic muscle disorders, heart muscle regeneration, and functional improvement after myocardial infarction or heart failure. ADSC have been shown as useful for neovascularization and, therefore, for ischemic diseases. Given their ability to differentiate into neurons, they are currently under investigation for neurological diseases, for brain injury, stroke, and peripheral nerve injury. Moreover, ADSC can be induced to become not only pancreatic/endocrine insulin-secreting cells, relevant for type 1 diabetes mellitus, but also hepatic cells, critical for chronic liver failure, hepatic regeneration, or even hepatocyte transplantation. Their immunomodulatory function, homing, and migratory patterns, as well as the approved clinical trials, suggest that these cells are efficient for treatment for several classes of autoimmune diseases and for treatment of GVDH (Graft versus Host Disease) and their application is safe [104]. On the whole, while the differentiation properties seem to be dependent on microenvironmental clues *in vivo*, the immunomodulatory effects appear to be rather intrinsic and present an attractive basis for the therapy of autoimmune and inflammatory diseases by systemic infusion.

Question remains, however, regarding the safety of ADSC application, and their long-term fate. Tumorigenic potential is especially worrisome. To date, any studies suggesting that ADSC have this ability are either inconclusive or have been retracted [105, 106]. In a recent study culture-expanded human-derived ADSC were applied to immunosuppressed mice. At one year, animals were no different in weight nor life span from controls and showed no signs of tumorigenesis

[107]. More over even if tumorogenesis is not proven tumor growth were reported in some studies through the enhancement of neovascularization [108, 109] or through the secretion of soluble signaling molecules [39].

In the light of the above, the stage seems to be set for clinicians to translate ADSC from the bench to the bedside. This process will involve "development" steps that fall outside of traditional "hypothesis-driven, mechanism-based" paradigm. Therefore, ADSC clinical potential represents an opportunity to coordinate basic research and translational efforts using the principles of evidenced-based medicine.

3.4. Regulatory Framework. In spite of the wealth of published evidence in animal models evaluating the safety and efficacy of ADSC, there are still open questions concerning the best ways to maintain quality when these cells are administered as medicinal products.

The European directives (Directive 2001/83/EC [110]; 2003/63/EC [111]; and Regulation 1394/2007 [112]) on human cell-based medicinal products acknowledges that, given their complexity, conventional clinical and nonclinical pharmacology, and toxicology studies may not be appropriate for cell-based medicinal products. Cell-based medicinal products can be considered as a single production, more similar to a graft than to a chemical drug and often involve cell samples of limited amount, mostly to be used in a patient-specific manner. The highly repetitive and controlled methods employed in the manufacture of cellular products are a critical factor since these products are partly defined by reference to their method of manufacture. Therefore, all the personnel, as well as the physical structures and the materials, involved in each step, must be in accordance with Good Manufacturing Practice and within an accepted quality system.

Different regulatory bodies around the world provide GMP guidance (EudraLex-Volume 4 GMP Guidelines [113]). The aim of all laws on cellular medicinal products is the guarantee of their overall safety, with a positive balance between risks and benefits for patients. The risk posed by the administration of a cell-based medicinal product, in fact, is highly dependent on the origin of the cells, the manufacturing process, the noncellular components, and on the specific therapeutic use. The following general risk criteria can be used in the estimation of the overall risk of the product: origin (autologous-allogeneic); ability to proliferate and differentiate; ability to initiate an immune response (as target or effector); level of cell manipulation; mode of administration (*ex vivo* perfusion, local, systemic); duration of exposure (short to permanent) and availability of clinical data on or experience with similar products (EMEA/CHMP/410869/2006) [114].

To reduce these risks, cell-based medicinal products intended for clinical use must be produced via a robust manufacturing process governed by a quality control sufficient to ensure consistent and reproducible final product. Structure layout and design must minimize the risk of errors and permit effective cleaning and maintenance in order to avoid cross-contamination, buildup of dust or dirt, and, in general, any adverse effect on the quality of products. The manufacture must be carried out in clean areas (class A in B, in C, and in D), entry to which is through airlocks for personnel and/or for equipment and materials. In order to meet "in operation" conditions, these areas must be designed to reach certain specified air-cleanliness levels in the "at rest" occupancy state. Clean rooms and clean air devices are routinely monitored in operation and the monitoring locations are based on a formal risk analysis study. Standard operating procedures (SOP) must be written and concern manufacturing and release of cell-based products, starting materials, traceability, and quality controls. Viral and TSE safety of the cells and raw materials have to be addressed to minimize the risk of contamination. When bovine serum is used, the recommendations of the Note for Guidance on the "Use of Bovine Serum in the Manufacture of Human Biological Medicinal Product" must be followed.

Moreover, cell viability has to be preserved in the final product but cell-based medicinal products cannot be sterilized at the end of the process: they have to be produced aseptically and their sterility must be proven. All cell products for human clinical applications must be free of bacterial, endotoxin, mycoplasma, and viral (B19, cytomegalovirus, Epstein-Barr virus, hepatitis B and C, human immunodeficiency viruses 1 and 2, and human T-cell leukemia viruses 1 and 2, EMEA/CHMP/410869/2006) [114] contamination. The adipose tissue donors may themselves be carriers of infectious agents and the possibility of viral infections has to be eliminated prior to sample acceptance.

Once produced, GMP grade products must be maintained in liquid nitrogen vapor phase storage containers, that, again, remove any risk of cross-contamination between individual containers.

Given the complexity of GMP requirements, regulatory compliance is challenging not only for cell-therapy laboratories but also for the entire scientific community. Regulatory agencies should establish the best criteria to guarantee safety of cell-based medicinal products and, on the other hand, should assure that all available treatments can reach the patients.

4. Discussion and Conclusions

The focus on regenerative medicine is increasing and cell therapy modalities are becoming more available; thus adult stem cells represent the most promising source of cell for plastic surgery and several of these therapies are potentially useful also in aesthetic applications. At the beginning, the clinicians believed that damaged human tissue could be replaced only with a transplant from a donor site or with alloplastic implants.

Concerning plastic surgery, the potential of using ADSC is endless, with a spectrum of treatment areas and types of reconstruction needed. From oncological reconstruction to aesthetic uses, each application can be individually tailored to the patient. Today we can use the stem cells obtained from bone marrow or adipose tissue and they could be used in several modalities. They can be cultured and expanded in good cell factories that process tissues for several weeks to generate enough cells for therapeutic use. This procedure

is expensive, heavily regulated and these limitations have stimulated interest on the therapeutic role of ADSC from processed lipoaspirate.

In laboratory and clinical experiences, you can observe the use of autologous cultured stem cells or of autologous fresh adipose tissue, or adipose tissue naturally aspirated enriched with a mixture of mesenchymal stem cells, or eventually enriched with platelet-derived growth factors [**?**]. As a biological indicator of the usefulness of the mixture of cells on the skin, Akita et al. have shown a dramatic healing of chronic and refractory wounds from irradiation [115, 116] or for diabetic wound treatment modalities [117]. Another application of ADSC is, as said, the contemporary transplantation with autologous fat, usually referred to as cell assisted lipotransfer or cells enriched fat grafting. It has been demonstrated by preclinical data that when ADSC were added to the fat grafts, the establishment of grafting is double. Clinical data, as noted by these authors, are promising [118, 119]. This method was employed mainly for difficult wounds fat grafting, as tissues irradiated, after-effects of burns, but also in breast augmentation, breast asymmetry, facial balance and be around the silicone breast implants or pocket after removal [120]. If there is a clear advantage to the application of fat grafting cells enriched versus regular fat in normal tissues recipients is yet to be determined. A gold standard does not exist for this particular technique and several studies and clinical trials are underway with several changes of the underlying technology. Even if it is notable that comparative studies in aesthetic surgery have not been carried out. Researchers and clinician need more data to be able to determine the ideal source of multipotential stem cells, and to decide the appropriate therapy for each patient.

Conflict of Interests

The authors declare that there is no competing interests.

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References

[1] C. W. Patrick Jr., "Tissue engineering strategies for adipose tissue repair," *Anatomical Record*, vol. 263, no. 4, pp. 361–366, 2001.

[2] S. R. Coleman, "Long-term survival of fat transplants: controlled demonstrations," *Aesthetic Plastic Surgery*, vol. 19, no. 5, pp. 421–425, 1995.

[3] D. A. Young and K. L. Christman, "Injectable biomaterials for adipose tissue engineering," *Biomedical Materials*, vol. 7, no. 2, Article ID 024104, 2012.

[4] J. M. Anderson, A. Rodriguez, and D. T. Chang, "Foreign body reaction to biomaterials," *Seminars in Immunology*, vol. 20, no. 2, pp. 86–100, 2008.

[5] S. Franz, S. Rammelt, D. Scharnweber, and J. C. Simon, "Immune responses to implants—a review of the implications for the design of immunomodulatory biomaterials," *Biomaterials*, vol. 32, no. 28, pp. 6692–6709, 2011.

[6] R. Langer and J. P. Vacanti, "Tissue engineering," *Science*, vol. 260, no. 5110, pp. 920–926, 1993.

[7] K. Okabe, Y. Yamada, K. Ito, T. Kohgo, R. Yoshimi, and M. Ueda, "Injectable soft-tissue augmentation by tissue engineering and regenerative medicine with human mesenchymal stromal cells, platelet-rich plasma and hyaluronic acid scaffolds," *Cytotherapy*, vol. 11, no. 3, pp. 307–316, 2009.

[8] J. R. Fuchs, B. A. Nasseri, and J. P. Vacanti, "Tissue engineering: a 21st century solution to surgical reconstruction," *Annals of Thoracic Surgery*, vol. 72, no. 2, pp. 577–591, 2001.

[9] E. Lavik and R. Langer, "Tissue engineering: current state and perspectives," *Applied Microbiology and Biotechnology*, vol. 65, no. 1, pp. 1–8, 2004.

[10] S. D. Lin, K. H. Wang, and A. P. Kao, "Engineered adipose tissue of predefined shape and dimensions from human adipose-derived mesenchymal stem cells," *Tissue Engineering—Part A*, vol. 14, no. 5, pp. 571–581, 2008.

[11] J. R. Mauney, T. Nguyen, K. Gillen, C. Kirker-Head, J. M. Gimble, and D. L. Kaplan, "Engineering adipose-like tissue in vitro and in vivo utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds," *Biomaterials*, vol. 28, no. 35, pp. 5280–5290, 2007.

[12] R. M. Shanti, S. Janjanin, W. J. Li et al., "In vitro adipose tissue engineering using an electrospun nanofibrous scaffold," *Annals of Plastic Surgery*, vol. 61, no. 5, pp. 566–571, 2008.

[13] B. Weiser, L. Prantl, T. E. O. Schubert et al., "In vivo development and long-term survival of engineered adipose tissue depend on in vitro precultivation strategy," *Tissue Engineering—Part A*, vol. 14, no. 2, pp. 275–284, 2008.

[14] M. S. Stosich, B. Bastian, N. W. Marion, P. A. Clark, G. Reilly, and J. J. Mao, "Vascularized adipose tissue grafts from human mesenchymal stem cells with bioactive cues and microchannel conduits," *Tissue Engineering*, vol. 13, no. 12, pp. 2881–2890, 2007.

[15] M. Neubauer, M. Hacker, P. Bauer-Kreisel et al., "Adipose tissue engineering based on mesenchymal stem cells and basic fibroblast growth factor in vitro," *Tissue Engineering*, vol. 11, no. 11-12, pp. 1840–1851, 2005.

[16] S. W. Cho, W. S. Kang, W. R. Jong, H. P. Moon, Y. C. Cha, and B. S. Kim, "Engineered adipose tissue formation enhanced by basic fibroblast growth factor and a mechanically stable environment," *Cell Transplantation*, vol. 16, no. 4, pp. 421–434, 2007.

[17] Y. S. Choi, S. N. Park, and H. Suh, "Adipose tissue engineering using mesenchymal stem cells attached to injectable PLGA spheres," *Biomaterials*, vol. 26, no. 29, pp. 5855–5863, 2005.

[18] A. V. Vashi, K. M. Abberton, G. P. Thomas et al., "Adipose tissue engineering based on the controlled release of fibroblast growth factor-2 in a collagen matrix," *Tissue Engineering*, vol. 12, no. 11, pp. 3035–3043, 2006.

[19] Y. Hiraoka, H. Yamashiro, K. Yasuda, Y. Kimura, T. Inamoto, and Y. Tabata, "In situ regeneration of adipose tissue in rat fat pad by combining a collagen scaffold with gelatin microspheres containing basic fibroblast growth factor," *Tissue Engineering*, vol. 12, no. 6, pp. 1475–1487, 2006.

[20] Y. Kimura, M. Ozeki, T. Inamoto, and Y. Tabata, "Adipose tissue engineering based on human preadipocytes combined with gelatin microspheres containing basic fibroblast growth factor," *Biomaterials*, vol. 24, no. 14, pp. 2513–2521, 2003.

[21] D. Von Heimburg, M. Kuberka, R. Rendchen, K. Hemmrich, G. Rau, and N. Pallua, "Preadipocyte-loaded collagen scaffolds with enlarged pore size for improved soft-tissue

engineering," *International Journal of Artificial Organs*, vol. 26, no. 12, pp. 1064–1076, 2003.

[22] E. Gentleman, E. A. Nauman, G. A. Livesay, and K. C. Dee, "Collagen composite biomaterials resist contraction while allowing development of adipocytic soft tissue in vitro," *Tissue Engineering*, vol. 12, no. 6, pp. 1639–1649, 2006.

[23] G. H. Altman, F. Diaz, C. Jakuba et al., "Silk-based biomaterials," *Biomaterials*, vol. 24, no. 3, pp. 401–416, 2003.

[24] J. H. Kang, J. M. Gimble, and D. L. Kaplan, "In vitro 3D model for human vascularized adipose tissue," *Tissue Engineering—Part A*, vol. 15, no. 8, pp. 2227–2236, 2009.

[25] M. Vermette, V. Trottier, V. Ménard, L. Saint-Pierre, A. Roy, and J. Fradette, "Production of a new tissue-engineered adipose substitute from human adipose-derived stromal cells," *Biomaterials*, vol. 28, no. 18, pp. 2850–2860, 2007.

[26] M. Vallée, J. F. Côté, and J. Fradette, "Adipose-tissue engineering: taking advantage of the properties of human adipose-derived stem/stromal cells," *Pathologie Biologie*, vol. 57, no. 4, pp. 309–317, 2009.

[27] L. E. Flynn, G. D. Prestwich, J. L. Semple, and K. A. Woodhouse, "Proliferation and differentiation of adipose-derived stem cells on naturally derived scaffolds," *Biomaterials*, vol. 29, no. 12, pp. 1862–1871, 2008.

[28] L. Flynn, G. D. Prestwich, J. L. Semple, and K. A. Woodhouse, "Adipose tissue engineering in vivo with adipose-derived stem cells on naturally derived scaffolds," *Journal of Biomedical Materials Research—Part A*, vol. 89, no. 4, pp. 929–941, 2009.

[29] A. Porzionato, M. M. Sfriso, V. Macchi et al., "Decellularization of rat and human omentum to develop novel scaffolds to be recellularized with adipose derived stem cells," *Italian Journal of Anatomy and Embryology*, vol. 116, no. 1, supplement, article 149, 2011.

[30] N. Torio-Padron, N. Baerlecken, A. Momeni, G. B. Stark, and J. Borges, "Engineering of adipose tissue by injection of human preadipocytes in fibrin," *Aesthetic Plastic Surgery*, vol. 31, no. 3, pp. 285–293, 2007.

[31] L. A. Solchaga, J. E. Dennis, V. M. Goldberg, and A. I. Caplan, "Hyaluronic acid-based polymers as cell carriers for tissue-engineered repair of bone and cartilage," *Journal of Orthopaedic Research*, vol. 17, no. 2, pp. 205–213, 1999.

[32] M. Halbleib, T. Skurk, C. de Luca, D. Von Heimburg, and H. Hauner, "Tissue engineering of white adipose tissue using hyaluronic acid-based scaffolds. I: in vitro differentiation of human adipocyte precursor cells on scaffolds," *Biomaterials*, vol. 24, no. 18, pp. 3125–3132, 2003.

[33] N. Kawaguchi, K. Toriyama, E. Nicodemou-Lena, K. Inou, S. Torii, and Y. Kitagawa, "De novo adipogenesis in mice at the site of injection of basement membrane and basic fibroblast growth factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 3, pp. 1062–1066, 1998.

[34] K. M. Abberton, S. K. Bortolotto, A. A. Woods et al., "Myogel, a novel, basement membrane-rich, extracellular matrix derived from skeletal muscle, is highly adipogenic in vivo and in vitro," *Cells Tissues Organs*, vol. 188, no. 4, pp. 347–358, 2008.

[35] E. K. Beahm, R. L. Walton, and C. W. Patrick Jr., "Progress in adipose tissue construct development," *Clinics in Plastic Surgery*, vol. 30, no. 4, pp. 547–558, 2003.

[36] D. L. Crandall, G. J. Hausman, and J. G. Kral, "A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives," *Microcirculation*, vol. 4, no. 2, pp. 211–232, 1997.

[37] M. E. Francis, S. Uriel, and E. M. Brey, "Endothelial cell-matrix interactions in neovascularization," *Tissue Engineering—Part B*, vol. 14, no. 1, pp. 19–32, 2008.

[38] J. H. Miner and P. D. Yurchenco, "Laminin functions in tissue morphogenesis," *Annual Review of Cell and Developmental Biology*, vol. 20, pp. 255–284, 2004.

[39] W. Zhu, L. Huang, Y. Li et al., "Mesenchymal stem cell-secreted soluble signaling molecules potentiate tumor growth," *Cell Cycle*, vol. 10, no. 18, pp. 3198–3207, 2011.

[40] S. Y. Song, H. M. Chung, and J. H. Sung, "The pivotal role of VEGF in adipose-derived-stem-cell-mediated regeneration," *Expert Opinion on Biological Therapy*, vol. 10, no. 11, pp. 1529–1537, 2010.

[41] A. Garten, S. Schuster, and W. Kiess, "The insulin-like growth factors in adipogenesis and obesity," *Endocrinology and Metabolism Clinics of North America*, vol. 41, no. 2, pp. 283–295, 2012.

[42] K. J. Cronin, A. Messina, K. R. Knight et al., "New murine model of spontaneous autologous tissue engineering, combining an arteriovenous pedicle with matrix materials," *Plastic and Reconstructive Surgery*, vol. 113, no. 1, pp. 260–269, 2004.

[43] M. Lovett, C. Cannizzaro, L. Daheron, B. Messmer, G. Vunjak-Novakovic, and D. L. Kaplan, "Silk fibroin microtubes for blood vessel engineering," *Biomaterials*, vol. 28, no. 35, pp. 5271–5279, 2007.

[44] L. Meinel, V. Karageorgiou, S. Hofmann et al., "Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds," *Journal of Biomedical Materials Research—Part A*, vol. 71, no. 1, pp. 25–34, 2004.

[45] Y. Wang, D. J. Blasioli, H. J. Kim, H. S. Kim, and D. L. Kaplan, "Cartilage tissue engineering with silk scaffolds and human articular chondrocytes," *Biomaterials*, vol. 27, no. 25, pp. 4434–4442, 2006.

[46] V. Zacchi, C. Soranzo, R. Cortivo, M. Radice, P. Brun, and G. Abatangelo, "In vitro engineering of human skin-like tissue," *Journal of Biomedical Materials Research*, vol. 40, no. 2, pp. 187–194, 1998.

[47] J. Aigner, J. Tegeler, P. Hutzler et al., "Cartilage tissue engineering with novel nonwoven structured biomaterial based on hyaluronic acid benzyl ester," *Journal of Biomedical Materials Research*, vol. 42, no. 2, pp. 172–181, 1998.

[48] J. H. Piasecki, K. A. Gutowski, K. M. Moreno, and G. L. Lahvis, "Purified viable fat suspended in matrigel improves volume longevity," *Aesthetic Surgery Journal*, vol. 28, no. 1, pp. 24–32, 2008.

[49] Y. H. Song, L. Prantl, and E. Alt, "Differentiation and plasticity of stem cells for tissue engineering," in *Tissue Engineering*, N. Pallua and C. V. Suschek, Eds., part 1, pp. 113–130, Springer, Berlin, Germany, 2011.

[50] A. Giordano, U. Galderisi, and I. R. Marino, "From the laboratory bench to the patient's bedside: an update on clinical trials with Mesenchymal Stem Cells," *Journal of Cellular Physiology*, vol. 211, no. 1, pp. 27–35, 2007.

[51] F. D. Watanabe, C. J. P. Mullon, W. R. Hewitt et al., "Clinical experience with a bioartificial liver in the treatment of severe liver failure: a phase I clinical trial," *Annals of Surgery*, vol. 225, no. 5, pp. 484–494, 1997.

[52] P. A. Zuk, M. Zhu, P. Ashjian et al., "Human adipose tissue is a source of multipotent stem cells," *Molecular Biology of the Cell*, vol. 13, no. 12, pp. 4279–4295, 2002.

[53] H. Eto, H. Suga, D. Matsumoto et al., "Characterization of structure and cellular components of aspirated and excised

adipose tissue," *Plastic and Reconstructive Surgery*, vol. 124, no. 4, pp. 1087–1097, 2009.

[54] J. K. Fraser, I. Wulur, Z. Alfonso, and M. H. Hedrick, "Fat tissue: an underappreciated source of stem cells for biotechnology," *Trends in Biotechnology*, vol. 24, no. 4, pp. 150–154, 2006.

[55] W. S. Kim, B. S. Park, J. H. Sung et al., "Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts," *Journal of Dermatological Science*, vol. 48, no. 1, pp. 15–24, 2007.

[56] J. Rehman, D. Traktuev, J. Li et al., "Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells," *Circulation*, vol. 109, no. 10, pp. 1292–1298, 2004.

[57] A. Miranville, C. Heeschen, C. Sengenès, C. A. Curat, R. Busse, and A. Bouloumié, "Improvement of postnatal neovascularization by human adipose tissue-derived stem cells," *Circulation*, vol. 110, no. 3, pp. 349–355, 2004.

[58] J. Gao, J. E. Dennis, R. F. Muzic, M. Lundberg, and A. I. Caplan, "The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion," *Cells Tissues Organs*, vol. 169, no. 1, pp. 12–20, 2001.

[59] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., "Multilineage potential of adult human mesenchymal stem cells," *Science*, vol. 284, no. 5411, pp. 143–147, 1999.

[60] A. I. Caplan, "Mesenchymal stem cells," *Journal of Orthopaedic Research*, vol. 9, no. 5, pp. 641–650, 1991.

[61] L. Casteilla, V. Planat-Bénard, B. Cousin et al., "Plasticity of adipose tissue: a promising therapeutic avenue in the treatment of cardiovascular and blood diseases?" *Archives des Maladies du Coeur et des Vaisseaux*, vol. 98, no. 9, pp. 922–926, 2005.

[62] M. J. Oedayrajsingh-Varma, S. M. van Ham, M. Knippenberg et al., "Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure," *Cytotherapy*, vol. 8, no. 2, pp. 166–177, 2006.

[63] B. Puissant, C. Barreau, P. Bourin et al., "Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells," *British Journal of Haematology*, vol. 129, no. 1, pp. 118–129, 2005.

[64] D. O. Traktuev, S. Merfeld-Clauss, J. Li et al., "A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks," *Circulation Research*, vol. 102, no. 1, pp. 77–85, 2008.

[65] P. A. Zuk, M. Zhu, H. Mizuno et al., "Multilineage cells from human adipose tissue: Iimplications for cell-based therapies," *Tissue Engineering*, vol. 7, no. 2, pp. 211–228, 2001.

[66] H. Mizuno and H. Hyakusoku, "Mesengenic potential and future clinical perspective of human processed lipoaspirate cells," *Journal of Nippon Medical School*, vol. 70, no. 4, pp. 300–306, 2003.

[67] J. M. Gimble, A. J. Katz, and B. A. Bunnell, "Adipose-derived stem cells for regenerative medicine," *Circulation Research*, vol. 100, no. 9, pp. 1249–1260, 2007.

[68] E. Arrigoni, S. Lopa, L. De Girolamo, D. Stanco, and A. T. Brini, "Isolation, characterization and osteogenic differentiation of adipose-derived stem cells: from small to large animal models," *Cell and Tissue Research*, vol. 338, no. 3, pp. 401–411, 2009.

[69] A. C. W. Zannettino, S. Paton, A. Arthur et al., "Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo," *Journal of Cellular Physiology*, vol. 214, no. 2, pp. 413–421, 2008.

[70] F. Guilak, K. E. Lott, H. A. Awad et al., "Clonal analysis of the differentiation potential of human adipose-derived adult stem cells," *Journal of Cellular Physiology*, vol. 206, no. 1, pp. 229–237, 2006.

[71] Y. A. Romanov, A. N. Darevskaya, N. V. Merzlikina, and L. B. Buravkova, "Mesenchymal stem cells from human bone marrow and adipose tissue: isolation, characterization, and differentiation potentialities," *Bulletin of Experimental Biology and Medicine*, vol. 140, no. 1, pp. 138–143, 2005.

[72] A. M. Bailey, S. Kapur, and A. J. Katz, "Characterization of adipose-derived stem cells: an update," *Current Stem Cell Research and Therapy*, vol. 5, no. 2, pp. 95–102, 2010.

[73] B. Lindroos, R. Suuronen, and S. Miettinen, "The potential of adipose stem cells in regenerative medicine," *Stem Cell Reviews and Reports*, vol. 7, no. 2, pp. 269–291, 2011.

[74] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[75] R. Taléns-Visconti, A. Bonora, R. Jover et al., "Hepatogenic differentiation of human mesenchymal stem cells from adipose tissue in comparison with bone marrow mesenchymal stem cells," *World Journal of Gastroenterology*, vol. 12, no. 36, pp. 5834–5845, 2006.

[76] J. A. Kode, S. Mukherjee, M. V. Joglekar, and A. A. Hardikar, "Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration," *Cytotherapy*, vol. 11, no. 4, pp. 377–391, 2009.

[77] D. Calderon, V. Planat-Benard, V. Bellamy et al., "Immune response to human embryonic stem cell-derived cardiac progenitors and adipose-derived stromal cells," *Journal of Cellular and Molecular Medicine*, vol. 16, no. 7, pp. 1544–1552, 2012.

[78] L. Cui, S. Yin, W. Liu, N. Li, W. Zhang, and Y. Cao, "Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E_2," *Tissue Engineering*, vol. 13, no. 6, pp. 1185–1195, 2007.

[79] M. J. Hoogduijn, M. Roemeling-van Rhijn, S. S. Korevaar, A. U. Engela, W. Weimar, and C. C. Baan, "Immunological aspects of allogeneic and autologous mesenchymal stem cell therapies," *Human Gene Therapy*, vol. 22, no. 12, pp. 1587–1591, 2011.

[80] A. M. Rodriguez, C. Elabd, E. Z. Amri, G. Ailhaud, and C. Dani, "The human adipose tissue is a source of multipotent stem cells," *Biochimie*, vol. 87, no. 1, pp. 125–128, 2005.

[81] P. Smith, W. P. Adams, A. H. Lipschitz et al., "Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival," *Plastic and Reconstructive Surgery*, vol. 117, no. 6, pp. 1836–1844, 2006.

[82] V. Van Harmelen, T. Skurk, K. Röhrig et al., "Effect of BMI and age on adipose tissue cellularity and differentiation capacity in women," *International Journal of Obesity*, vol. 27, no. 8, pp. 889–895, 2003.

[83] B. M. Schipper, K. G. Marra, W. Zhang, A. D. Donnenberg, and J. P. Rubin, "Regional anatomic and age effects on cell function of human adipose-derived stem cells," *Annals of Plastic Surgery*, vol. 60, no. 5, pp. 538–544, 2008.

[84] V. Zachar, J. G. Rasmussen, and T. Fink, "Isolation and growth of adipose tissue-derived stem cells," *Methods in Molecular Biology (Clifton, N.J.)*, vol. 698, pp. 37–49, 2011.

[85] T. Fink, J. G. Rasmussen, P. Lund, L. Pilgaard, K. Soballe, and V. Zachar, "Isolation and expansion of adipose-derived

stem cells for tissue engineering," *Frontiers in Bioscience (Elite edition)*, vol. 3, pp. 256–263, 2011.

[86] S. K. Williams, S. McKenney, and B. E. Jarrell, "Collagenase lot selection and purification for adipose tissue digestion," *Cell Transplantation*, vol. 4, no. 3, pp. 281–289, 1995.

[87] J. M. Gimble, B. A. Bunnell, E. S. Chiu, and F. Guilak, "Concise review: adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation," *Stem Cells*, vol. 29, no. 5, pp. 749–754, 2011.

[88] K. C. Hicok and M. H. Hedrick, "Automated isolation and processing of adipose-derived stem and regenerative cells," *Methods in Molecular Biology (Clifton, N.J.)*, vol. 702, pp. 87–105, 2011.

[89] J. P. DeLany, Z. E. Floyd, S. Zvonic et al., "Proteomic analysis of primary cultures of human adipose-derived stem cells," *Molecular and Cellular Proteomics*, vol. 4, no. 6, pp. 731–740, 2005.

[90] B. A. Bunnell, M. Flaat, C. Gagliardi, B. Patel, and C. Ripoll, "Adipose-derived stem cells: isolation, expansion and differentiation," *Methods*, vol. 45, no. 2, pp. 115–120, 2008.

[91] M. Dhanasekaran, S. Indumathi, J. S. Rajkumar, and D. Sudarsanam, "Effect of high glucose on extensive culturing of mesenchymal stem cells derived from subcutaneous fat, omentum fat and bone marrow," *Cell Biochemistry and Function*. In press.

[92] P. J. Amos, S. K. Kapur, P. C. Stapor et al., "Human adipose-derived stromal cells accelerate diabetic wound healing: impact of cell formulation and delivery," *Tissue Engineering—Part A*, vol. 16, no. 5, pp. 1595–1606, 2010.

[93] M. Banerjee and R. R. Bhonde, "Application of hanging drop technique for stem cell differentiation and cytotoxicity studies," *Cytotechnology*, vol. 51, no. 1, pp. 1–5, 2006.

[94] L. Aust, B. Devlin, S. J. Foster et al., "Yield of human adipose-derived adult stem cells from liposuction aspirates," *Cytotherapy*, vol. 6, no. 1, pp. 7–14, 2004.

[95] A. H. F. Bakker, F. M. H. Van Dielen, J. W. M. Greve, J. A. Adam, and W. A. Buurman, "Preadipocyte number in omental and subcutaneous adipose tissue of obese individuals," *Obesity Research*, vol. 12, no. 3, pp. 488–498, 2004.

[96] C. T. Gomillion and K. J. L. Burg, "Stem cells and adipose tissue engineering," *Biomaterials*, vol. 27, no. 36, pp. 6052–6063, 2006.

[97] X. Wei, Z. Du, L. Zhao et al., "IFATS collection: the conditioned media of adipose stromal cells protect against hypoxia-ischemia-induced brain damage in neonatal rats," *Stem Cells*, vol. 27, no. 2, pp. 478–488, 2009.

[98] W. S. Kim, B. S. Park, and J. H. Sung, "The wound-healing and antioxidant effects of adipose-derived stem cells," *Expert Opinion on Biological Therapy*, vol. 9, no. 7, pp. 879–887, 2009.

[99] W. S. Kim, B. S. Park, S. H. Park, H. K. Kim, and J. H. Sung, "Antiwrinkle effect of adipose-derived stem cell: activation of dermal fibroblast by secretory factors," *Journal of Dermatological Science*, vol. 53, no. 2, pp. 96–102, 2009.

[100] G. E. Kilroy, S. J. Foster, X. Wu et al., "Cytokine profile of human adipose-derived stem cells: expression of angiogenic, hematopoietic, and pro-inflammatory factors," *Journal of Cellular Physiology*, vol. 212, no. 3, pp. 702–709, 2007.

[101] T. G. Ebrahimian, F. Pouzoulet, C. Squiban et al., "Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 4, pp. 503–510, 2009.

[102] T. G. Ebrahimian, F. Pouzoulet, C. Squiban et al., "Suppression of hepatocyte growth factor production impairs the ability of adipose-derived stem cells to promote ischemic tissue revascularization," *Stem Cells*, vol. 25, no. 12, pp. 3234–3243, 2007.

[103] E. Y. Lee, Y. Xia, W. S. Kim et al., "Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF," *Wound Repair and Regeneration*, vol. 17, no. 4, pp. 540–547, 2009.

[104] B. Fang, Y. P. Song, L. M. Liao, Q. Han, and R. C. Zhao, "Treatment of severe therapy-resistant acute graft-versus-host disease with human adipose tissue-derived mesenchymal stem cells [3]," *Bone Marrow Transplantation*, vol. 38, no. 5, pp. 389–390, 2006.

[105] D. Rubio, J. Garcia-Castro, M. C. Martín et al., "Spontaneous human adult stem cell transformation," *Cancer Research*, vol. 65, no. 8, pp. 3035–3039, 2005.

[106] R. De La Fuente, A. Bernad, J. Garcia-Castro, M. C. Martin, and J. C. Cigudosa, "Retraction: spontaneous human adult stem cell transformation," *Cancer Research*, vol. 70, no. 16, p. 6682, 2010.

[107] Z. M. MacIsaac, H. Shang, H. Agrawal, N. Yang, A. Parker, and A. J. Katz, "Long-term in-vivo tumorigenic assessment of human culture-expanded adipose stromal/stem cells," *Experimental Cell Research*, vol. 318, no. 4, pp. 416–423, 2012.

[108] Y. Liu, Z.-P. Han, S.-S. Zhang et al., "Effects of inflammatory factors on mesenchymal stem cells and their role in the promotion of tumor angiogenesis in colon cancer," *Journal of Biological Chemistry*, vol. 286, no. 28, pp. 25007–25015, 2011.

[109] K. Suzuki, R. Sun, M. Origuch et al., "Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization," *Molecular Medicine*, vol. 17, no. 7-8, pp. 579–587, 2011.

[110] http://www.edctp.org/fileadmin/documents/ethics/DIRECTIVE_200183EC_OF_THE_EUROPEAN_PARLIAMENT.pdf.

[111] http://ec.europa.eu/health/files/eudralex/vol-1/dir_2003_63/dir_2003_63_en.pdf.

[112] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:en:PDF.

[113] http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm.

[114] http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003898.pdf.

[115] A. Casadei, E. Agolli, M. C. Vicari Sottosanti, B. Bovani, and S. Cinti :, "Clinical use of ADSC in plastic surgery: case report," in *Proceedings of the International Conference on Adipose Tissue (ICAT '11)*, Venice, Italy, December 2011.

[116] S. Akita, K. Akino, A. Hirano, A. Ohtsuru, and S. Yamashita, "Mesenchymal stem cell therapy for cutaneous radiation syndrome," *Health Physics*, vol. 98, no. 6, pp. 858–862, 2010.

[117] S. Akita, K. Akino, A. Hirano, A. Ohtsuru, and S. Yamashita, "Noncultured autologous adipose-derived stem cells therapy for chronic radiation injury," *Stem Cells International*, vol. 2010, Article ID 532704, 8 pages, 2010.

[118] M. V. Karaaltin and S. Baghaki, "Adipose derived regenerative cell therapy for treating a diabetic wound: a case report," *Wounds*, vol. 23, no. 12, pp. 536–542, 2011.

[119] M. Zhu, Z. Zhou, Y. Chen et al., "Supplementation of fat grafts with adipose-derived regenerative cells improves long-term graft retention," *Annals of Plastic Surgery*, vol. 64, no. 2, pp. 222–228, 2010.

[120] D. Matsumoto, K. Sato, K. Gonda et al., "Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection," *Tissue Engineering*, vol. 12, no. 12, pp. 3375–3382, 2006.

[121] S. Lendeckel, A. Jödicke, P. Christophis et al., "Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: case report," *Journal of Cranio-Maxillofacial Surgery*, vol. 32, no. 6, pp. 370–373, 2004.