Human Adipose Stem Cells: Current Clinical Applications

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Summary: Adipose-derived stem cells are multipotent cells that can easily be extracted from adipose tissue, are capable of expansion in vitro, and have the capacity to differentiate into multiple cell lineages, which have the potential for use in regenerative medicine. However, several issues need to be studied to determine safe human use. For example, there are questions related to isolation and purification of adipose-derived stem cells, their effect on tumor growth, and the enforcement of U.S. Food and Drug Administration regulations. Numerous studies have been published, with the interest in the potential for regenerative medicine continually growing. Several clinical trials using human adipose stem cell therapy are currently being performed around the world, and there has been a rapid evolution and expansion of their number. The purpose of this article was to review the current published basic science evidence and ongoing clinical trials involving the use of adipose-derived stem cells in plastic surgery and in regenerative medicine in general. The results of the studies and clinical trials using adipose-derived stem cells reported in this review seem to be promising not only in plastic surgery but also in a wide variety of other specialties. Nevertheless, those reported showed disparity in the way adipose-derived stem cells were used. Further basic science experimental studies with standardized protocols and larger randomized trials need to be performed to ensure safety and efficacy of adipose-derived stem cells use in accordance with U.S. Food and Drug Administration guidelines. (*Plast. Reconstr. Surg.* 129: 1277, 2012.)

Human adipose-derived stem cells are multipotent autologous mesenchymal stem cells. These multipotent cells are recognized as a potential regenerative tool that may be beneficial in a wide variety of medical therapies in reconstructive surgery and in a multitude of other medical disciplines.[1] The clinical potential of adipose-derived stem cells has proven to be a source of considerable enthusiasm and scientific curiosity in academic circles and more recently commercially as an emerging business opportunity. Clinicians and patients alike have high expectations that adipose-derived stem cells may well be the answer to curing many recalcitrant diseases or reconstructing anatomical defects.

As clinical applications using adipose-derived stem cells have been increasingly reported, there has been growing concern, generating criticism that clinical practices using adipose-derived stem cells have not been substantiated by rigorous scientific evidence. To address some of these concerns, in May of 2011, the American Society of Plastic Surgeons and the American Society for Aesthetic Plastic Surgery published a joint position statement concerning stem cells.[2] This document reported that the scientific evidence was very limited in terms of assessing the safety and efficacy of stem cell therapies in aesthetic medicine. This statement included the recommendations of the Task Force, which stated in general terms that stem cell procedures should be performed in compliance with U.S. Food and Drug Administration regulatory guidelines, and that the preparation and processing of adipose-derived stem cells should be conducted in accordance with current good manufacturing practice guidelines.[3]

The aim of this report is to collate the results of published reports that specifically look at the basic science behind adipose-derived stem cell

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therapies from different units across the globe. In so doing, we hope to provide a comprehensive review of the current literature across all medical disciplines that describe the clinical uses of adipocyte-derived stem cells in clinical medicine.

ADIPOSE-DERIVED STEM CELLS: DEFINITION, EXTRACTION, AND PROPERTIES

Adipose-derived stem cells were identified as such by Zuck et al. in 2001; these authors defined the stem cell characteristics of adipose-derived stem cells by their ability to differentiate into several mesenchymal lineages.[4] Adipose-derived stem cell extraction from adipose tissue requires a multistep laboratory-based process. The majority of laboratories use collagenases that generate a cell pellet following centrifugation that has been termed the stromal vascular fraction. The stromal vascular fraction consists of multiple cell types, including circulating blood cells, fibroblasts, pericytes, endothelial cells, and adipose-derived stem cells.[5] Adipose-derived stem cells can be isolated from the stromal vascular fraction through culturing on plastic as, unlike the other cell types in the stromal vascular fraction, they adhere to plastic. In addition, they can be identified by means of fluorescence-activated cell sorting, as they have several specific surface markers: $CD73^+$, $CD90^+$, $CD105^+$, $CD45^-$, $CD34^-$, CD14 or CD11b, $CD79^-$ or $CD19^-$, and HLA-DR[6] (Fig. 1).

Adipose-derived stem cells possess the ability to readily be expanded in vitro and the capacity to undergo adipogenic, osteogenic, chondrogenic, and myogenic (cardiomyocyte and skeletal myocyte) differentiation.[4] Neurogenic differentiation potential has also been described in vitro,[7–10] as has pancreatic endocrine phenotype expressing insulin,[11] hepatic,[12,13] and endothelial differentiation (Fig. 2).[14]

The metabolic properties of adipose-derived stem cells (angiogenic, antioxidative, immunotolerance) have been demonstrated in bench experiments and increasingly in preclinical models.[15–24] First, adipose-derived stem cells secrete a favorable cytokine profile that is angiogenic, immunosuppressive, and antioxidative.[25] The cytokine profile of adipose-derived stem cells contains large amounts of vascular endothelial growth factor, transforming growth factor-β, hepatocyte growth factor, platelet-derived growth factor, placental growth factor, and basic fibroblast growth factor, which explains their impressive angiogenic capacity and their ability to induce tissue neovascularization.[16]

Second, adipose-derived stem cells have been shown to be immune-privileged because of a lack of human leukocyte antigen–DR expression and the suppression of the proliferation of activated allogenic lymphocytes.[26,27] Adipose-derived stem cells have also been shown to inhibit the production of inflammatory cytokines and to stimulate the production of antiinflammatory cytokines.[28,29]

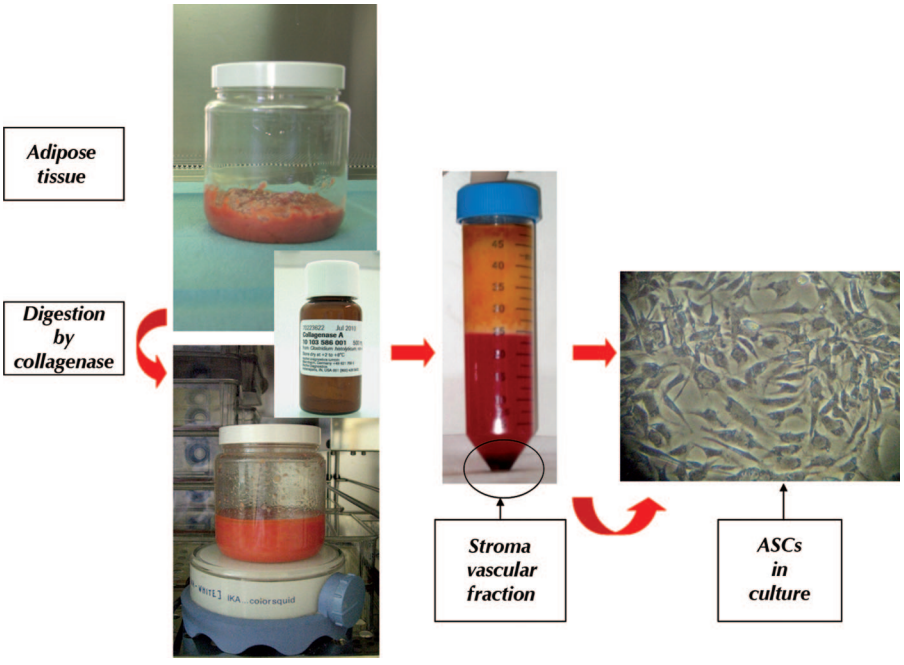

Fig. 1. Extraction and culture of adipose-derived stem cells (*ASCs*).

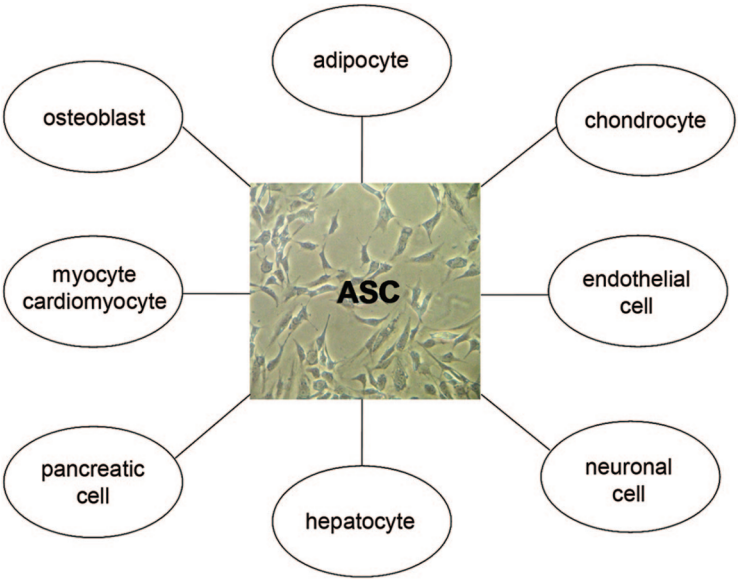

Fig. 2. Differentiation of adipose-derived stem cells (*ASC*) into several lineages.

The immunomodulatory properties of adipose-derived stem cells have been demonstrated in vitro and in vivo.[30–33] The correlations of the data sets derived from these models are currently being tested in several clinical studies to be reviewed later.

There is still concern regarding adipose-derived stem cell use in a clinical setting. In a recent review, published in March of 2011, Locke et al. emphasized that the literature revealed considerable uncertainty about the true clinical potential of adipose-derived stem cells.[34] According to these authors, first, the differentiation of adipose-derived stem cells into cell lineages in vivo has not been conclusively demonstrated in many studies because of the use of rather simplistic approaches to the confirmation of differentiation. An example of this would be neurogenic differentiation of adipose-derived stem cells.[35] Second, adipose-derived stem cells prepared from human liposuction from different studies differ in purity and molecular phenotype, with many studies using cell preparations that are likely to contain heterogeneous populations of cells, making it uncertain whether adipose-derived stem cells themselves are responsible for effects observed. They concluded that the full clinical potential of adipose-derived stem cells awaits much deeper investigation of their fundamental biology.

The immunologic and angiogenic properties of adipose-derived stem cells raise the question of the relation of these cells with promoting cancer. Several contradictory studies have been published, with some reports demonstrating that adipose-derived stem cells could promote tumor growth.[36–41] Conversely, others report that adipose-derived stem cells could have a tumor-suppressive effect.[42–44] The answer to that question remains unknown, and further studies are necessary to determine the effect of adipose-derived stem cells on tumor formation.

Lastly, the production of a clinically acceptable grade of adipose-derived stem cells requires careful assessment of the risks and benefits. It is important to identify and control all possible molecules that may affect the efficacy of the adipose-derived stem cell preparation, which should be performed in accordance with current good manufacturing practice guidelines.

U.S. FOOD AND DRUG ADMINISTRATION REGULATIONS, GOOD MANUFACTURING PRACTICE, AND THE EUROPEAN POSITION

The U.S. Food and Drug Administration has developed a regulatory framework based on three areas[45]:

1. Prevention of use of contaminated tissues or cells.
2. Prevention of inadequate handling or processing that may damage or contaminate those tissues or cells.
3. Clinical safety of all tissues or cells that may be processed, used for functions other than normal function, combined with components other than tissues, or used for metabolic purpose.

In the United States, adipose-derived stem cells are considered in the context of human cells, tissues, or cellular and tissue–based products, and their production must comply with Current Good Tissue Practice requirements, under the Code of Federal Regulations, Title 21, Part 1271.[46] Human cells, tissues, or cellular and tissue–based products are defined as articles containing or consisting of human cells or tissues that are intended for implantation, infusion, or transfer into a human recipient. The essential Current Good Tissue Practice requirements are related to preventing the introduction, transmission, or spread of communicable disease by human cells, tissues, or cellular and tissue–based products.[3]

Two levels of regulation apply: for a low level of risk, a human cell, tissue, or cellular and tissue–based product is regulated solely under Section 361 of the Public Health System Act.[46] This is true if it meets all the following criteria (Part 1271.10):

1. The human cell, tissue, or cellular and tissue–based product is minimally manipulated.
2. The human cell, tissue, or cellular and tissue–based product is intended for homologous use only.
3. The manufacture of the human cell, tissue, or cellular and tissue–based product does not involve the combination of the cells or tissues with another article.
4. That the human cell, tissue, or cellular and tissue–based product does not have a systemic effect and is not dependent on metabolic activity of living cells for its primary function or the human cell, tissue, or cellular and tissue–based product has a systemic effect or is dependent on the metabolic activity of living cells for its primary function, and is for autologous use.

In this case, the U.S. Food and Drug Administration sanctioned clinical trials as an investigational new drug and a formal U.S. Food and Drug Administration approval process for the specific therapy is not required. For a higher level of risk (more than minimal manipulation, e.g., ex vivo expansion, combination with nontissue components, or transduction), the human cell, tissue, or cellular and tissue–based product is considered a drug, device, or biological product and is regulated under Section 351 of the Public Health System Act. Consequently, at the higher level of risk, to introduce adipose-derived stem cells or deliver them for clinical use, as a drug, a valid biologics license must be in effect. Such licenses are issued only after the product has been shown to be safe and efficacious for its intended use. While in the development stage, such products may be distributed for clinical use for humans only if the sponsor has an investigational new drug application in effect as specified by U.S. Food and Drug Administration regulations (Title 21, Code of Federal Regulations, Part 312).[47]

In Europe, adipose-derived stem cells are considered Advanced Therapy Medicinal Products, as defined by the European Regulation (European Commission) 1394/2007, which contains rules for "authorization, supervision, and technical requirements regarding the summary of products characteristics, labeling, and the package leaflet of Advanced Therapy Medicinal Products that are prepared following industrials methods and in academic institutions."[47] This regulation refers to the European good manufacturing process rules.[48]

The process of converting research-based protocols using adipose-derived stem cells into a safe manufacturing process that is good manufacturing process–compliant requires protocols that have had careful consideration of all the risks and benefits for the patient end user. In particular, Sensebé et al. stated that the following parameters should be considered: sources and collection methods, cell seeding, proliferation rate, and culture medium. They went on to identify steps for quality control that must be carried out at cell harvest and during the various phases of adipose-derived stem cell production.[49]

Furthermore, automated devices for separating adipose stem cells are regulated as class III medical devices by the U.S. Food and Drug Administration.[50] Currently, no such device is approved for human use in the United States. These are considered as research tools and should only be used under and approved by the Product Development Protocol.[51] Because of the rigors of safe, reproducible, quality-controlled adipose-derived stem cell production as required by the U.S. Food and Drug Administration, it is easy to see why the use of adipose-derived stem cells in clinical trials or for clinical applications is still very rare in the United States.

CLINICAL APPLICATIONS OF ADIPOSE-DERIVED STEM CELLS: PUBLISHED LITERATURE AND ONGOING CLINICAL TRIALS

This section reports the different publications found concerning the clinical use of adipose-derived stem cells and also the clinical trials currently being performed around the world. These appli-

cations were organized into two areas: plastic surgery and the other medical specialties.

In a review published in June of 2011, Lindroos et al. found 18 clinical trials concerning the use of adipose-derived stem cells in regenerative medicine.[52] A search performed on www.clinicaltrials.gov with the search term "adipose stem cell therapy," performed in August of 2011, revealed 33 studies based on adipose-derived stem cell therapy, which demonstrates the rapid evolution and expansion of clinical use of adipose-derived stem cells. Five clinical trials were found for the plastic surgery area (Table 1), and 28 were found in the other specialties (Table 2).

Table 3 represents the location of the clinical trials in the world. Spain and Korea are the two leaders in this area, with 10 studies performed in each country. Only three trials are currently being performed in the United States, which is attributable to the high level of exigency of the U.S. Food and Drug Administration regulations.

PUBLISHED CLINICAL APPLICATIONS AND CLINICAL TRIALS IN PLASTIC SURGERY

Plastic surgery is a field where the clinical use of adipose-derived stem cells is well developed. The cells are usually in the form of autologous stromal vascular fraction cells or noncultured adipose-derived stem cells, with one treatment given in a local immediate administration mainly in combination with fat grafts. Within the literature, adipose-derived stem cells have been used or studied in three main areas: soft-tissue augmentation, wound healing, and tissue engineering.

Published Clinical Applications

Soft-Tissue Augmentation

Yoshimura et al.[53–55] reported several studies using the cell-assisted lipotransfer technique for treatment of facial lipoatrophy, cosmetic breast augmentation, or immediate breast augmentation after breast implant removal (Table 4).[53–61] The principle of this technique is to enrich the fat grafting material with stromal vascular fraction. In their breast augmentation study, the authors stated that "augmentation effects were apparently increased with the cell-assisted lipotransfer technique compared with patients who underwent traditional lipoinjection," but no comparative control group was used.[54] In their facial lipoatrophy study, one half of the face was treated with cell-assisted lipotransfer and the contralateral side was treated by the traditional lipoinjection

Table 1. Current Clinical Trials in Plastic Surgery

Trials in Plastic Surgery*	Phase	Status	No. of ASCs	Evaluation	Location
Study of autologous adipose-derived stem cell transplantation in patients with lipodystrophy	I	Active; 5 patients enrolled	Local lipoinjection enriched with autologous ASCs	Clinical evaluation of the transplanted area (volume improvement)	Brazil
Effect of human adipose tissue–derived MSCs in Romberg disease	II	Completed; 5 patients enrolled	Intramuscular infusion of autologous ASCs (10^7 cells/500 μl)	Evaluation of the volume change of fatty layer using 3D camera	Korea
Safety and efficacy of autologous cultured adipocytes in patients with depressed scar (AdipoCell)†	II and III	Completed; 36 patients enrolled	From 0.11–4.63 $\times$ 10^7 autologous ASCs into each scar	Evaluation of the scar improvement and the safety (adverse effect)	Korea
Study of autologous fat enhanced with ASCs transplanted to reconstruct breast deformities after lumpectomy (RESTORE-2)	IV	Completed; estimated enrollment, 71	Local injection of fat graft enriched with autologous ASCs	Evaluation of satisfaction with functional and cosmetic results, improvement in overall breast deformity measured at 12 mo compared to baseline	Belgium, Italy, Spain, United Kingdom
Study to demonstrate the effectiveness of Antria cell preparation process in extraction of SVF from adipose tissue	I	Recruiting; estimated enrollment, 4	—	Evaluation of the device	United States

ASC, adipose-derived stem cell; MSCs, mesenchymal stem cells; 3D, three-dimensional; SVF, stromal vascular fraction.
*All clinical trials taken from www.clinicaltrials.gov.
†Kim M, Kim I, Lee SK, Bang SI, Lim SY. Clinical trial of autologous differentiated adipocytes from stem cells derived from human adipose tissue. *Dermatol Surg.* 2011;37:750–759.

Table 2. Current Clinical Trials in Other Specialties

Trial*	Phase	Status	No. of ASCs	Evaluation	Location
Digestive diseases					
Crohn disease fistula					
Study AdipoPlus: efficacy and safety of autologous ASCs for Crohn fistula	I	Completed; 9 patients enrolled	Local injection of autologous ASCs	Closure of the fistula and adverse events	Korea
Study ALOREVA: efficacy and safety of allogenic ASCs for treatment of rectovaginal fistulas in Crohn disease	I–IIa	Recruiting; 10 patients enrolled	Intralesional injection of $20–40 \times 10^6$ allogenic ASCs	Efficacy (closure of the fistula), adverse events	Spain
Phase II AdipoPlus	II	Recruiting; estimated enrollment, 40	Local injection of 10^7 autologous ASCs	Efficacy and adverse events	Korea
Study of efficacy and safety of expanded allogenic ASCs for treatment of complex perianal fistulas in perianal Crohn disease	I–IIa	Completed; 24 patients enrolled	Intralesional injection of $20–40 \times 10^6$ allogenic ASCs	Adverse events, closure of the fistulas, reduction of the number of draining fistulas	Spain
Treatment of fistulous Crohn disease by autologous ASCs	I–II	Recruiting; estimated enrollment, 15	Autologous ASCs	Healing efficiency, quality of life, change of systemic Crohn disease, characterization of the cell product	Spain
Follow-up of autologous cultured ASCs for the Crohn fistula (AdipoPlus)	III	Recruiting; estimated enrollment, 40	Autologous ASCs	Durability of efficacy and safety of AdipoPlus injection 6 mo after injection	Korea
Safety and efficacy of ASCs to treat complex perianal fistulas in Crohn disease (FATT)	III	Completed; 56 patients	Intralesional injection of $20–40 \times 10^6$ autologous ASCs	Randomized, single-blind, placebo-controlled, multicenter study	Austria, Spain, The Netherlands
Complex perianal fistula not associated with Crohn disease					
Nonsurgical treatment of complex perianal fistula with ASCs†	II	Completed; 50 patients	Local injection of autologous ASCs	Multicenter, randomized study; complete closure at week 8; no recurrence after 1 yr	Spain
FATT-1 trial: efficacy and safety of ASCs to treat perianal fistulas not associated with Crohn disease	III	Completed; 214 enrolled	Intralesional injection of $20–40 \times 10^6$ autologous ASCs	Closure of the fistulas, adverse effects	Spain, Germany, United Kingdom
Long-term safety of FATT-1 trial	III	Completed; 148 patients enrolled	Intralesional injection of $20–40 \times 10^6$ autologous ASCs	Adverse events, closure of the fistula	Spain
Efficacy and safety of ASCs on the complex fistula patients	II	Recruiting; estimated enrollment, 40	Local injection of $1–2 \times 10^7$ autologous ASCs	Complete closure of the fistula, investigator's satisfaction, adverse events	Korea
Fecal incontinence					
Safety of autologous ASCs for fecal incontinence	I	Terminated	Local injection of ASCs	Wexner score evaluation, adverse events	Korea
Autoimmune diseases					
Safety and efficacy of autologous ASCs in patients with diabetes mellitus type 1	I–II	Estimated enrollment, 30	Intravenous injection of SVF cells from 100 ml of fat	Lowering of insulin dependence and HbA1C, increase of C-peptide levels	Philippines
Safety and efficacy of autologous ASCs in type 2 diabetics	I–II	34 patients enrolled	Intravenous injection of SVF cells from 100 ml of fat	Lowering of blood glucose, decrease of hyperglycemic medication dosages, well-being of patients	Philippines

(*Continued*)

Table 2. (*Continued*)

Trial*	Phase	Status	No. of ASCs	Evaluation	Location
Autologous transplantation of ASCs in Buerger disease	I–II	Recruiting; estimated enrollment, 18	Intramuscular injection of 5×10^6 cells/kg	Treadmill walking distance, safety evaluation	Korea
ASCs in patients with chronic graft-versus-host disease	I	Recruiting; estimated enrollment, 30	Intravenous injection of $1–3 \times 10^6$ allogenic ASCs/kg	Adverse events, reduction of corticosteroid treatment, disease-free survival	Spain
Cardiovascular diseases					
PRECISE trial: ASCs in treatment of nonrevascularizable myocardium	I	Active; estimated enrollment, 36	Injection into left ventricle of autologous ASCs	Major adverse cardiac and cerebral events, feasibility, cardiac function	Denmark, The Netherlands, Spain
APOLLO trial: ASCs in treatment of patients with ST-segment elevation myocardial infarction	I	Active; estimated enrollment, 48	Autologous ASCs	Major adverse cardiac and cerebral events, feasibility, cardiac function	The Netherlands, Spain
ASCs in diabetic patients with chronic limb ischemia	I–II	Recruiting; estimated enrollment, 36	Intraarterial femoral injection of $0.5–1 \times 10^6$ cells/kg autologous ASCs	Adverse events, clinical outcome	Spain
ASCs for critical limb ischemia for diabetic patients	I–II	Recruiting; estimated enrollment, 36	Intraarterial femoral injection of $0.5–2 \times 10^6$ cells/kg autologous ASCs	Angiographic assessment of neovasculogenesis, adverse events	Spain
ACELLDream (patients with peripheral vascular disease not amenable to bypass or angioplasty)	I–II	Recruiting; estimated enrollment, 15	Intramuscular injection of 100×10^6 autologous ASCs	Adverse events	France
Feasibility study of ASC-coated ePTFE vascular graft	I–II	Recruiting; estimated enrollment, 60	Autologous ASC–coated vascular graft	Graft patency, limb salvage, wound healing, rest pain	United States
Treatment of diabetic lower extremity wounds and venous stasis ulcers with lipoaspirate injection	I–II	Estimated enrollment, 250	Local injection of adipose tissue containing ASCs	Wound healing	United States
Skeletal regeneration					
ASCs in patients with degenerative arthritis	I–II	Recruiting; estimated enrollment, 18	Intraarticular injection of 1×10^7 to 1×10^8/3 ml autologous ASCs	Safety, WOMAC index	Korea
ASCROD	I–II	Started; estimated enrollment, 30	Local implantation of 10^6 autologous ASCs/cm^2 lesion	Hyaline cartilage production, clinical and functional evaluation, adverse events	Spain
Development of bone grafts using ASCs and different scaffolds	I	Recruiting; estimated enrollment, 100	—	Bone formation	Switzerland
Neurologic diseases					
Autologous ASCs in patients with spinal cord injury‡	I	Completed; 8 patients	Intravenous injection of 4×10^8 autologous SVFcells	Safety evaluation	Korea
Autologous ASCs in patients with secondary progressive multiple sclerosis	I–II	Recruiting; estimated enrollment, 30	Intravenous injection of $1–4 \times 10^6$ autologous ASCs	Safety and tolerability of ASC injection	Spain

ASCs, adipose-derived stem cells; FATT, fistula advanced therapy trial; SVF, stromal vascular fraction; PRECISE, Phase III Randomized Evaluation of Convection Enhanced Delivery of Il13-Pe38qqr with Survival Endpoint; APOLLO, Acute Myocardial Infarction and Coronary Artery Disease; ACELLDream, Adipose Cell Derived Regenerative Endothelial Angiogenic Medicine; ASCROD, Autologous ASCs vs. Chondrocytes for the Repair of Chondral Knee Defects; WOMAC, Western Ontario and McMaster Universities Arthritis Index; ePTFE, expanded polytetrafluoroethylene.

*All clinical trials were taken from www.clinicaltrials.gov.

†Garcia-Olmo D, Herreros D, Pascual M, et al. Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: A comparison of protocols with and without cell expansion. *Int J Colorectal Dis.* 2009;24:27–30.

‡Ra JC, Shin IS, Kim SH, et al. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells Dev.* 2011;20:1297–1308.

Table 3. Repartition of Clinical Trials in the World

Country	No. of Clinical Trials
Americas	4
United States	3
Brazil	1
Asia	12
Korea	10
Philippines	2
Europe	17
Spain	10 (+5 multicenter studies with: Belgium, Italy, United Kingdom, Austria, The Netherlands, Germany, Denmark)
Switzerland	1
France	1
Total	33

technique.[55] They reported that transplanted adipose tissue was absorbed faster in the non–cell-assisted lipotransfer group compared with the cell-assisted lipotransfer–treated group over the long term.

In 2011, two additional noncontrolled trials using the cell-assisted lipotransfer strategy were published. In the first, by Tiryaki et al., the authors observed not only a better graft take but also a subjective gradual regeneration of the skin overlying the graft, in 29 patients undergoing soft-tissue augmentation.[56] They stated that the technique was particularly successful in secondary cases that had been previously treated with fat grafting without significant improvement. The second study, by Kamakura and Ito, reported 20 cases of breast augmentation using the cell-assisted lipotransfer technique and concluded that this technique is a safe and effective method for breast augmentation.[57]

In 2011, Kim et al. published the result of a clinical trial involving 31 patients treated with adipose-derived stem cells that were differentiated into mature adipocytes for facial depressed scars, but again with no comparative control group.[58] Adipose-derived stem cells were isolated from adipose tissue harvested by liposuction, expanded in culture, and differentiated into mature adipocytes, which they termed "AdipoCell" (Table 1). AdipoCell preparations were injected subcutaneously into the depressed scars, and the volume of each scar was measured using three-dimensional scans. They observed 74.6 percent mean recovery in volume when the received dose of AdipoCell was equivalent to three-eighths of the cell volume of the defect and that the volume was stable at 1 year after treatment. They concluded that this technique represented a new safe and effective therapy for soft-tissue augmentation.

Wound Healing

In 2007, Rigotti et al. published a study concerning 20 patients undergoing therapy for side effects of radiation treatment with severe symptoms and irreversible functional damage from radiation wounds.[59] Fat was harvested by manual liposuction and centrifuged, and the purified lipoaspirate was injected into the irradiated treatment site. An improvement of tissue wound healing was observed, and the authors postulated that the lipoaspirate was rich in native adipose-derived stem cells that contributed to the observed effect.

In 2010, Akita et al. reported on a case of one patient with an intractable wound in the sacrococcygeal region secondary to radiation therapy.[60] The wound healed with a combination treatment of a human recombinant basic fibroblast growth factor, artificial skin substitute, and autologous adipose-derived stem cells, some of which were injected into the wound. However, the true effect

Table 4. Clinical Applications of Adipose-Derived Stem Cells in Plastic Surgery

References	No. of Patients Treated	Dose of ASCs and Administration
Soft-tissue augmentation		
Yoshimura et al.[53]	15	263.5 ml of fat enriched with SVF injected into each breast
Yoshimura et al.[54]	40	272.7 ml of fat enriched with SVF injected into each breast
Yoshimura et al.[55]		133 ml fat injection in the non-CAL group, 100 ml fat enriched with SVF in the CAL group
Tiryaki et al.[56]	29	10–390 ml of fat enriched with SVF (CAL) by local administration
Kamakura and Ito[57]	20	240 ml of fat enriched with SVF in each breast (CAL)
Kim et al.[58]	31	$0.11–4.63 \times 10^7$ autologous ASCs into each scar
Wound healing		
Rigotti et al.[59]	20	$7.4 \pm 3.6 \times 10^5$ autologous SVF in each wound (60–80 ml of fat tissue)
Akita et al.[60]	1	3.8×10^7 autologous SVF into the wound
Tissue engineering		
Stillaert et al.[61]	12	$0.67–1.4 \times 10^6$ ASCs per scaffold
Total	174	

ASCs, adipose-derived stem cells; SVF, stromal vascular fraction; CAL, cell-assisted lipotransfer.

of adipose-derived stem cells was confounded by the presence of other treatment modalities.

Tissue Engineering

The use of adipose-derived stem cells seeded onto natural or synthesized scaffolds has been reported as a method of soft-tissue engineering or tissue regeneration. However, the clinical results to date remain inconclusive. One clinical study was performed by Stillaert et al. in 2008, who subcutaneously implanted hyaluronic acid scaffolds seeded with adipose-derived stem cells into 12 volunteers.[61] The authors observed no new adipose tissue formation, and concluded that this was attributable to a deficient angiogenic response to sustain the long-term adipose-derived stem cell viability and no adverse effect.

Clinical Trials

Five clinical trials involving adipose-derived stem cell therapies were found, and three studies have been completed (Table 1). However, not all published results are available. The main focus of these clinical trials was soft-tissue augmentation.

A phase I study of autologous adipose-derived stem cell transplantation is currently ongoing in Brazil in patients with lipodystrophy. Lipoinjection enriched with adipose-derived stem cells is being performed, and five subjects are enrolled. The primary outcome measure is the volume improvement of the transplanted area.

A phase II study in Korea is examining the effect of adipose-derived stem cells in Romberg disease, and five subjects are enrolled. The primary outcome measure is the evaluation of the volume change of the fatty layer using three-dimensional image analysis.

Phase II and III studies were studying the safety and efficacy of autologous cultured adipose-derived stem cells in patients with depressed scars, and 36 subjects were enrolled to receive cultured autologous adipose-derived stem cells that had been differentiated into mature adipocytes (AdipoCell). The primary outcome measures were to assess scar improvement and safety.

A phase IV postmarket study, RESTORE-2, examined autologous fat enhanced with adipose-derived stem cells for reconstructing breast deformities after lumpectomy. Primary outcome measures were patient and physician satisfaction assessments with functional and cosmetic improvement in overall breast deformity correction at 12 months. This study was conducted in Belgium, Italy, Spain, and the United Kingdom.

In the United States, a phase II proof-of-concept study by Antria is currently recruiting to demonstrate the effectiveness of their digestive enzymes for human use (Antria Cell Preparation Process) for extraction of stromal vascular fraction from adipose tissue.

In summary, the patients treated with adipose-derived stem cells in plastic surgery represent, thus far, 174 published cases to our knowledge (Table 4). One hundred twenty-one patients are or have been enrolled in clinical trials (Table 1). In all published cases, no major adverse effects have been reported. The results were encouraging in soft-tissue augmentation and in wound healing.

PUBLISHED CLINICAL APPLICATIONS AND CLINICAL TRIALS IN OTHER SPECIALTIES

Published Clinical Applications

To date, a wide variety of specialties have used adipose-derived stem cell therapy, but the number of patients who have been treated with adipose-derived stem cells is still very limited and represents 115 patients around the world (Table 5).[62–84] Most of the publications retrieved are case reports and noncontrolled studies of level 4/5 evidence, and results, although encouraging, have not been subject to the scientific rigors of a controlled trial.

From 2006 to 2011, Fang et al. used allogenic adipose-derived stem cells in the treatment of hematologic and immunologic disorders: graft-versus-host disease, idiopathic thrombocytopenic purpura, or pure red cell aplasia.[62–67] In each case, patients received intravenous infusion of allogenic adipose-derived stem cells isolated from adipose tissue of healthy donors. No adverse effects after the treatment were observed, and significant improvements were documented with recovery from graft-versus-host disease and pure red cell aplasia, and remission in the idiopathic thrombocytopenic purpura cases. These results provide evidence that the immunomodulatory effects of adipose-derived stem cells may be used in treating immunologic disorders.

In diabetes mellitus type 1, in 2008, Trivedi et al. treated five patients with allogenic adipose-derived stem cells cultured and differentiated into insulin-making mesenchymal stem cells transfused mixed with unfractionated autologous cultures of bone marrow.[68] No adverse effects were reported, all subjects were reported to be healthier and gaining weight, and biological markers were also improved. The authors concluded that adipose-derived stem cell therapy may be a solution for the treatment of insulinopenic patients.

Table 5. Clinical Applications of Adipose-Derived Stem Cells in Other Specialties

Specialties	References	No. of Patients Treated	Dose of ASCs and Administration
Hematologic and immunologic disorders	Fang et al.[62–67]	14	1–2 × 10^6 allogenic ASCs/kg, IV
Diabetes mellitus	Trivedi et al.[68]	5	3.15 × 10^6 allogenic ASCs injected by intraportal infusion under general anesthesia during minilaparotomy
	Vanikar et al.[69]	11	
Digestive diseases	Garcia-Olmo et al.[70–75]	63	3 × 10^6 to 2 × 10^7 autologous ASCs into the fistula
Autoimmune diseases	Ichim et al.[76]	1	53 × 10^6 autologous SVF in two IV infusions
	Riordan et al.[77]	3	25–75 × 10^6 autologous SVF IV
Tracheomediastinal fistula	Alvarez et al.[78]	1	4.9 × 10^6 autologous SVF into the fistula cavity
Bone tissue repair	Lendeckel et al.[79]	1	295 × 10^6 autologous SVF
	Mesimäki et al.[80]	1	13 × 10^6 autologous ASCs
	Taylor[81]	1	28 ml of solid fraction from fresh autologous lipoaspirate
	Pak[82]	4	10 cm^3 autologous SVF
Urologic disorder	Yamamoto et al.[83]	2	2.4–3.2 × 10^7 autologous SVF into urethral sphincter
Neurologic disease	Ra et al.[84]	8	4 × 10^8 autologous ASCs IV
Total		115	

ASCs, adipose-derived stem cells; IV, intravenously; SVF, stromal vascular fraction.

In 2010, Vanikar et al. reported the results obtained on 11 diabetic patients using the same treatment.[69] Again, no adverse events were reported and a gradual decrease in insulin requirements was noted. The authors concluded that easy and repeatable access to adipose tissue provided a clear advantage over isolation of mesenchymal stem cells from bone marrow for the treatment of diabetes mellitus by stem cell therapy.

From 2003 to 2010, García-Olmo et al. published several articles concerning the treatment of complex perianal or enterocutaneous fistulas, performing phase I and II clinical trials. These included patients with digestive fistulas associated or not with Crohn disease using autologous adipose-derived stem cells isolated mixed with fibrin glue and injected into the fistulous tract.[70–75] In all the studies, no adverse effects were reported and a significant healing rate was observed in patients who received adipose-derived stem cells (71 percent of healing compared with 16 percent). They concluded that adipose-derived stem cell therapy associated with fibrin glue is a safe and effective method of treating complex perianal fistula.

Rheumatoid arthritis treatments were examined in a case report in 2010 by Ichim et al. of a 67-year-old woman.[76] The patient was treated by intravenous infusions of autologous stromal vascular fraction cells isolated from a liposuction procedure. The authors observed no side effects, and the patient reported a considerable resolution of her pain joint and stiffness, with a decrease in rheumatoid factor. They concluded that adipose-derived stem cell therapy may be a treatment for rheumatoid arthritis and postulated that this was because of the immune-tolerance induced by adipose-derived stem cells.

In 2009, Riordan et al. reported the treatment of three multiple sclerosis patients with intravenous infusions of autologous stromal vascular fraction cells with multiple intrathecal and intravenous infusions of allogenic CD34 and mesenchymal stem cells within a 10-day period.[77] No adverse effects were documented, and by 3 months, all patients reported significant improvement of their symptoms. The authors concluded that further clinical evaluation of autologous stromal vascular fraction cells is warranted in autoimmune conditions.

In 2008, Alvarez et al. reported the case of a 67-year-old man suffering from lung cancer complicated with tracheomediastinal fistula and treated by autologous adipose-derived stem cells mixed with fibrin glue, injected into the fistula during bronchoscopy.[78] The patient's recovery was uneventful, and epithelialization of the fistula was observed after 3 months. One year after treatment, the fistula was closed, and 2 years after stem cell therapy, the patient was in complete remission.

There are four published case reports regarding bone tissue repair. In 2004, Lendeckel et al. published the case of a 7-year-old girl suffering from widespread calvarial defects after severe head injury.[79] The patient was treated with a combination of cancellous bone grafts from the

ilium and autologous stromal vascular fraction cells obtained from adipose tissue. Postoperative healing was uneventful and the clinical follow-up demonstrated symmetrical calvarial contour. At 3 months postoperatively, the computed tomographic scan showed a marked ossification in the defect areas.

In 2009, Mesimäki et al. reported the case of a 65-year-old patient who underwent a hemimaxillectomy because of a large keratocyst.[80] The patient's reconstruction was performed using a preformed titanium cage filled with autologous cultured adipose-derived stem cells, combined with synthetic bioresorbable beta–tricalcium phosphate granules. No adverse effects were reported, and bone regeneration was observed by biopsy. They concluded that the presence of adipose-derived stem cells may have enhanced the osteogenic and angiogenic conditions of the construct in vivo.

In 2010, Taylor published the case of a 14-year-old boy suffering from Treacher Collins syndrome whose severe biorbitozygomatic hypoplasia was treated with tissue-engineered bone using a combination of sculpted bone allograft, bone morphogenetic protein-2, periosteal grafts, and autologous fresh adipose-derived stem cells.[81] At 4 months, computed tomographic scanning showed complete bone reconstruction of the bilateral zygomas, and 6 months after surgery, a biopsy specimen showed lamellar bone with small marrow elements. The authors concluded that that type of engineered construct may provide an alternative method to both osteocutaneous free flaps and large structural allografts.

In 2011, Pak reported two cases of patients suffering from osteonecrosis of the femoral head and two cases of patients suffering from knee osteoarthritis.[82] All the patients were treated by a combination of percutaneously injected autologous adipose-derived stem cells, hyaluronic acid, platelet-rich plasma, and calcium chloride. At 3 months, in all cases, pain and mobilization were improved. Magnetic resonance imaging scans showed a significant filling of bone defects, with a possibility of bone matrix formation at the site of osteonecrosis and a significant increase in the thickness and the height of meniscus cartilage. The authors conclude that these good results can be explained either by a direct differentiation of adipose-derived stem cells or by the trophic effects of adipose-derived stem cells on existing tissues.

In 2010, Yamamoto et al. reported two cases of patients with stress urinary incontinence, which is a distressing complication of radical prostatectomy, who were treated with autologous adipose-derived stem cells isolated using the Celution System (Cytori Therapeutics, Inc., San Diego, Calif.) and injected (mixed with adipose tissue) in the external urethral sphincter under endoscopic vision.[83] The authors observed no adverse effects. Urinary incontinence improved progressively after 2 weeks, up to 12 weeks. Sphincter function of the urethra was improved in both cases, and magnetic resonance imaging showed a bulking effect at the site of injection. They concluded that adipose-derived stem cell therapy was a safe and feasible treatment for stress urinary incontinence.

In 2011, Ra et al. published a study of eight patients suffering from spinal cord injury who were treated with intravenous infusions of autologous adipose-derived stem cells.[84] The authors observed no serious adverse effects. At 12 weeks, motor function was improved in four patients. The authors concluded that they could not determine the efficacy of adipose-derived stem cell therapy because of their small patient group and the short follow-up period.

In summary, these studies reported no major adverse effects after treatment by adipose-derived stem cells, and the results were promising in the 115 published cases (Table 5). The characteristics of adipose-derived stem cell therapy in these specialties were as follows:

1. Adipose-derived stem cells used were allogenic or autologous; stromal vascular fraction cells or cultured adipose-derived stem cells.
2. In some cases, additive treatments (e.g., bone marrow, growth factor, fibrin glue) were used.
3. The administration doses were variable, as was the number of adipose-derived stem cells per dose.
4. Adipose-derived stem cells were administered systemically (intravenous infusion) or locally (intralesional injection).

Clinical Trials

Twenty-eight clinical trials using adipose and/or adipose-derived stem cells were listed; eight of these studies have been completed. The studies are separated by topic, clinical phase, primary site location, and study status (Table 2). The trials have been organized into five categories: digestive disease, autoimmune disease, cardiovascular disease, skeletal regeneration, and neurologic disorder.

In summary, adipose-derived stem cells have been used in a wide variety of ways:

1. Stromal vascular fraction cells or cultured adipose-derived stem cells.
2. Autologous or allogenic.
3. Varied doses and methods of administration.

Some clinical trials were based on immunologic or angiogenic properties of adipose-derived stem cells, for example, trials concerning the treatment of autoimmune diseases, limb ischemia, or diabetic wounds in the lower extremity. Other trials use differentiation of adipose-derived stem cells into several lineages to study their use in the treatment of degenerative arthritis, cardiac insufficiency, or spinal cord injury. Of the eight trials already completed, to our knowledge, only two have published results,[73,84] which are described in the previous section, and in both of those, there have been no adverse effects, and encouraging outcomes were reported.

CONCLUSIONS

The important role of adipose-derived stem cells in regenerative medicine is now coming under closer scrutiny. This is because adipose-derived stem cells are easily available and demonstrate several interesting properties, and evidence from preclinical studies suggests potential clinical promise in many medical disciplines. From this review, it can be noted that there is no standard protocol for adipose-derived stem cell use or clinical application in terms of type of cells used (stromal vascular fraction cells or cultured and purified adipose-derived stem cells). In addition, there is no consensus on the number of cells required per dose or treatment or how many treatments are required before an improved clinical outcome can be documented. Consequently, further basic science experimental studies with standardized protocols and larger randomized controlled trials need to be performed to ensure the safety and efficacy of adipose-derived stem cells in accordance with U.S. Food and Drug Administration guidelines. This review aims to make plastic surgeons aware, because of their unique privileged access to adipose tissue, of the development of adipose-derived stem cell therapies not only within plastic surgery but also, as evidenced by this review, the more frequent use in other medical specialties.

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REFERENCES

1. Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: Roles in adipose tissue remodeling and potential use for soft tissue augmentation. *Regen Med.* 2009;4:265–273.
2. ASAPS/ASPS position statement on stem cells and fat grafting. *Aesthet Surg J.* 2011;31:716–717.
3. U.S. Food and Drug Administration. Draft guidance for industry: Current good tissue practice (CGTP) and additional requirements for manufacturers of human cells, tissues, and cellular and tissue-based products (HCT/Ps). Available at: http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm073366.htm. Accessed October 6, 2011.
4. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng.* 2001;7:211–228.
5. Brown SA, Levi B, Lequeux C, Wong VW, Mojallal A, Longaker MT. Basic science review on adipose tissue for clinicians. *Plast Reconstr Surg.* 2010;126:1936–1946.
6. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315–317.
7. Ashjian PH, Elbarbary AS, Edmonds B, et al. In vitro differentiation of human processed lipoaspirate cells into early neural progenitors. *Plast Reconstr Surg.* 2003;111:1922–1931.
8. Fujimura J, Ogawa R, Mizuno H, Fukunaga Y, Suzuki H. Neural differentiation of adipose-derived stem cells isolated from GFP transgenic mice. *Biochem Biophys Res Commun.* 2005;333:116–121.
9. Safford KM, Hicok KC, Safford SD, et al. Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochem Biophys Res Commun.* 2002;294:371–379.
10. Safford KM, Safford SD, Gimble JM, Shetty AK, Rice HE. Characterization of neuronal/glial differentiation of murine adipose-derived adult stromal cells. *Exp Neurol.* 2004;187:319–328.
11. Timper K, Seboek D, Eberhardt M, et al. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun.* 2006;341:1135–1140.
12. Banas A, Teratani T, Yamamoto Y, et al. Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. *Hepatology* 2007;46:219–228.
13. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. *Biochem Biophys Res Commun.* 2005;328:258–264.
14. Planat-Benard V, Silvestre JS, Cousin B, et al. Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. *Circulation* 2004;109:656–663.
15. Moon MH, Kim SY, Kim YJ, et al. Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cell Physiol Biochem.* 2006;17:279–290.
16. Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: A critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci.* 2007;48:15–24.
17. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 2004;109:1292–1298.
18. Kim WS, Park BS, Kim HK, et al. Evidence supporting antioxidant action of adipose-derived stem cells: Protection of human dermal fibroblasts from oxidative stress. *J Dermatol Sci.* 2008;49:133–142.

19. González MA, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 2009;136:978–989.
20. Nakagami H, Maeda K, Morishita R, et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. *Arterioscler Thromb Vasc Biol.* 2005;25:2542–2547.
21. Kang JW, Kang KS, Koo HC, Park JR, Choi EW, Park YH. Soluble factors-mediated immunomodulatory effects of canine adipose tissue-derived mesenchymal stem cells. *Stem Cells Dev.* 2008;17:681–693.
22. Neels JG, Thinnes T, Loskutoff DJ. Angiogenesis in an in vivo model of adipose tissue development. *FASEB J.* 2004;18:983–985.
23. Kondo K, Shintani S, Shibata R, et al. Implantation of adipose-derived regenerative cells enhances ischemia-induced angiogenesis. *Arterioscler Thromb Vasc Biol.* 2009;29:61–66.
24. Eto H, Suga H, Inoue K, et al. Adipose injury-associated factors mitigate hypoxia in ischemic tissues through activation of adipose-derived stem/progenitor/stromal cells and induction of angiogenesis. *Am J Pathol.* 2011;178:2322–2332.
25. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res.* 2007;100:1249–1260.
26. Aust L, Devlin B, Foster SJ, et al. Yield of human adipose-derived adult stem cells from liposuction aspirates. *Cytotherapy* 2004;6:7–14.
27. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM. Surface protein characterization of human adipose tissue-derived stromal cells. *J Cell Physiol.* 2001;189:54–63.
28. Cui L, Yin S, Liu W, Li N, Zhang W, Cao Y. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E2. *Tissue Eng.* 2007;13:1185–1195.
29. Niemeyer P, Vohrer J, Schmal H, et al. Survival of human mesenchymal stromal cells from bone marrow and adipose tissue after xenogenic transplantation in immunocompetent mice. *Cytotherapy* 2008;10:784–795.
30. Puissant B, Barreau C, Bourin P, et al. Immunomodulatory effect of human adipose tissue-derived adult stem cells: Comparison with bone marrow mesenchymal stem cells. *Br J Haematol.* 2005;129:118–129.
31. Yañez R, Lamana ML, Garcia-Castro J, Colmenero I, Ramírez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006;24:2582–2591.
32. González MA, Gonzalez-Rey E, Rico L, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum.* 2009;60:1006–1019.
33. Constantin G, Marconi S, Rossi B, et al. Adipose-derived mesenchymal stem cells ameliorate chronic experimental autoimmune encephalomyelitis. *Stem Cells* 2009;27:2624–2635.
34. Locke M, Feisst V, Dunbar PR. Concise review: Human adipose-derived stem cells. Separating promise from clinical need. *Stem Cells* 2011;29:404–411.
35. Kompisch KM, Lange C, Steinemann D, et al. Neurogenic transdifferentiation of human adipose-derived stem cells? A critical protocol reevaluation with special emphasis on cell proliferation and cell cycle alterations. *Histochem Cell Biol.* 2010;134:453–468.
36. Zimmerlin L, Donnenberg AD, Rubin JP, Bassse P, Landreneau RJ, Donnenberg VS. Regenerative therapy and cancer: In vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. *Tissue Eng Part A* 2011;17:93–106.
37. Lin G, Yang R, Banie L, et al. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. *Prostate* 2010;70:1066–1073.
38. Prantl L, Muehlberg F, Navone NM, et al. Adipose tissue-derived stem cells promote prostate tumor growth. *Prostate* 2010;70:1709–1715.
39. Zhang Y, Daquinag A, Traktuev DO, et al. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. *Cancer Res.* 2009;69:5259–5266.
40. Muehlberg FL, Song YH, Krohn A, et al. Tissue-resident stem cells promote breast cancer growth and metastasis. *Carcinogenesis* 2009;30:589–597.
41. Yu JM, Jun ES, Bae YC, Jung JS. Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo. *Stem Cells Dev.* 2008;17:463–473.
42. Cousin B, Ravet E, Poglio S, et al. Adult stromal cells derived from human adipose tissue provoke pancreatic cancer cell death both in vitro and in vivo. *PLoS One* 2009;4:e6278.
43. Grisendi G, Bussolari R, Cafarelli L, et al. Adipose-derived mesenchymal stem cells as stable source of tumor necrosis factor-related apoptosis-inducing ligand delivery for cancer therapy. *Cancer Res.* 2010;70:3718–3729.
44. Kucerova L, Altanerova V, Matuskova M, Tyciakova S, Altaner C. Adipose tissue-derived human mesenchymal stem cells mediated prodrug cancer gene therapy. *Cancer Res.* 2007;67:6304–6313.
45. Liras A. Future research and therapeutic applications of human stem cells: General, regulatory, and bioethical aspects. *J Transl Med.* 2010;8:131.
46. U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21, Part 1271: Human cells, tissues, and cellular and tissue-based products. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271. Accessed October 6, 2011.
47. Sensebé L, Krampera M, Schrezenmeier H, Bourin P, Giordano R. Mesenchymal stem cells for clinical application. *Vox Sang.* 2010;98:93–107.
48. European Commission. EudraLex, Volume 4. Good manufacturing practice (GMP) guidelines. 2011. Available at: http://ec.europa.eu/health/documents/eudralex/vol-4/index_en.htm. Accessed October 6, 2011.
49. Sensebé L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. *Hum Gene Ther.* 2011;22:19–26.
50. U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21. Part 860: Medical device classification procedures. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=860.3. Accessed October 6, 2011.
51. U.S. Food and Drug Administration. CFR - Code of Federal Regulations Title 21. Part 814: Premarket approval of medical devices. Available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=814. Accessed October 6, 2011.
52. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev.* 2011;7:269–291.
53. Yoshimura K, Asano Y, Aoi N, et al. Progenitor-enriched adipose tissue transplantation as rescue for breast implant complications. *Breast J.* 2010;16:169–175.
54. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, Harii K. Cell-assisted lipotransfer for cosmetic breast augmentation:

Supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg.* 2008;32:48–55; discussion 56–57.
55. Yoshimura K, Sato K, Aoi N, et al. Cell-assisted lipotransfer for facial lipoatrophy: Efficacy of clinical use of adipose-derived stem cells. *Dermatol Surg.* 2008;34:1178–1185.
56. Tiryaki T, Findikli N, Tiryaki D. Staged stem cell-enriched tissue (SET) injections for soft tissue augmentation in hostile recipient areas: A preliminary report. *Aesthetic Plast Surg.* 2011;35:965–971.
57. Kamakura T, Ito K. Autologous cell-enriched fat grafting for breast augmentation. *Aesthetic Plast Surg.* 2011;35:1022–1030.
58. Kim M, Kim I, Lee SK, Bang SI, Lim SY. Clinical trial of autologous differentiated adipocytes from stem cells derived from human adipose tissue. *Dermatol Surg.* 2011;37:750–759.
59. Rigotti G, Marchi A, Galie M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg.* 2007;119:1409–1422; discussion 1423–1424.
60. Akita S, Akino K, Hirano A, Ohtsuru A, Yamashita S. Noncultured autologous adipose-derived stem cells therapy for chronic radiation injury. *Stem Cells Int.* 2010;2010:532704.
61. Stillaert FB, Di Bartolo C, Hunt JA, et al. Human clinical experience with adipose precursor cells seeded on hyaluronic acid-based spongy scaffolds. *Biomaterials* 2008;29:3953–3959.
62. Fang B, Song YP, Liao LM, Han Q, Zhao RC. Treatment of severe therapy-resistant acute graft-versus-host disease with human adipose tissue-derived mesenchymal stem cells. *Bone Marrow Transplant.* 2006;38:389–390.
63. Fang B, Song Y, Lin Q, et al. Human adipose tissue-derived mesenchymal stromal cells as salvage therapy for treatment of severe refractory acute graft-vs.-host disease in two children. *Pediatr Transplant.* 2007;11:814–817.
64. Fang B, Song Y, Zhao RC, Han Q, Lin Q. Using human adipose tissue-derived mesenchymal stem cells as salvage therapy for hepatic graft-versus-host disease resembling acute hepatitis. *Transplant Proc.* 2007;39:1710–1713.
65. Fang B, Song YP, Li N, Li J, Han Q, Zhao RC. Resolution of refractory chronic autoimmune thrombocytopenic purpura following mesenchymal stem cell transplantation: A case report. *Transplant Proc.* 2009;41:1827–1830.
66. Fang B, Song Y, Li N, Li J, Han Q, Zhao RC. Mesenchymal stem cells for the treatment of refractory pure red cell aplasia after major ABO-incompatible hematopoietic stem cell transplantation. *Ann Hematol.* 2009;88:261–266.
67. Fang B, Mai L, Li N, Song Y. Favorable response of chronic refractory immune thrombocytopenic purpura to mesenchymal stem cells. *Stem Cells Dev.* 2012;21:497–502.
68. Trivedi HL, Vanikar AV, Thakker U, et al. Human adipose tissue-derived mesenchymal stem cells combined with hematopoietic stem cell transplantation synthesize insulin. *Transplant Proc.* 2008;40:1135–1139.
69. Vanikar AV, Dave SD, Thakkar UG, Trivedi HL. Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: A novel therapy for insulin-dependent diabetes mellitus. *Stem Cells Int.* 2010;2010:582382.
70. García-Olmo D, García-Arranz M, García LG, et al. Autologous stem cell transplantation for treatment of rectovaginal fistula in perianal Crohn's disease: A new cell-based therapy. *Int J Colorectal Dis.* 2003;18:451–454.
71. García-Olmo D, García-Arranz M, Herreros D, et al. A phase I clinical trial of the treatment of Crohn's fistula by adipose mesenchymal stem cell transplantation. *Dis Colon Rectum* 2005;48:1416–1423.
72. Garcia-Olmo D, Garcia-Arranz M, Herreros D. Expanded adipose-derived stem cells for the treatment of complex perianal fistula including Crohn's disease. *Expert Opin Biol Ther.* 2008;8:1417–1423.
73. Garcia-Olmo D, Herreros D, Pascual I, et al. Expanded adipose-derived stem cells for the treatment of complex perianal fistula: A phase II clinical trial. *Dis Colon Rectum* 2009;52:79–86.
74. Garcia-Olmo D, Herreros D, Pascual M, et al. Treatment of enterocutaneous fistula in Crohn's Disease with adipose-derived stem cells: A comparison of protocols with and without cell expansion. *Int J Colorectal Dis.* 2009;24:27–30.
75. Garcia-Olmo D, Herreros D, De-La-Quintana P, et al. Adipose-derived stem cells in Crohn's rectovaginal fistula. *Case Report Med.* 2010;2010:961758.
76. Ichim TE, Harman RJ, Min WP, et al. Autologous stromal vascular fraction cells: A tool for facilitating tolerance in rheumatic disease. *Cell Immunol.* 2010;264:7–17.
77. Riordan NH, Ichim TE, Min WP, et al. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *J Transl Med.* 2009;7:29.
78. Alvarez PD, García-Arranz M, Georgiev-Hristov T, García-Olmo D. A new bronchoscopic treatment of tracheomediastinal fistula using autologous adipose-derived stem cells. *Thorax* 2008;63:374–376.
79. Lendeckel S, Jödicke A, Christophis P, et al. Autologous stem cells (adipose) and fibrin glue used to treat widespread traumatic calvarial defects: Case report. *J Craniomaxillofac Surg.* 2004;32:370–373.
80. Mesimäki K, Lindroos B, Törnwall J, et al. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. *Int J Oral Maxillofac Surg.* 2009;38:201–209.
81. Taylor JA. Bilateral orbitozygomatic reconstruction with tissue-engineered bone. *J Craniofac Surg.* 2010;21:1612–1614.
82. Pak J. Regeneration of human bones in hip osteonecrosis and human cartilage in knee osteoarthritis with autologous adipose-tissue-derived stem cells: A case series. *J Med Case Reports* 2011;5:296.
83. Yamamoto T, Gotoh M, Hattori R, et al. Periurethral injection of autologous adipose-derived stem cells for the treatment of stress urinary incontinence in patients undergoing radical prostatectomy: Report of two initial cases. *Int J Urol.* 2010;17:75–82.
84. Ra JC, Shin IS, Kim SH, et al. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells Dev.* 2011;20:1297–1308.