Review

Off-label use of adipose-derived stem cells

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ABSTRACT

Background: Adipose-derived stem cells (ASCs) have a broad range of clinical applications. The ease of cell harvest and high yield with minimal donor-site morbidity makes adipose tissue an ideal source of stem cells. Further, the multi-lineage potential of these cells present significant opportunities within the field of tissue engineering, with studies successfully demonstrating their ability to produce a range of tissue types.

Materials and methods: Literature review of publications on the use of ASCs, in the context of current European and US regulations.

Results: According to European and US regulations, many clinical trials reported in literature to date could be considered off-label.

Conclusion: In Europe, clinical trials involving cultured ASCs and/or the use of collagenase, which causes changes in the structural and functional properties of stem cells, and/or ASCs application in non-homologous tissue, should be considered off-label. ASCs should be non-cultured, isolated mechanically, and used only in the subcutaneous tissue.

1. Introduction

Recent advances in regenerative medicine, particularly the discovery of multipotent, easily accessible stem cells such as adipose-derived stem cells (ASCs), have provided the opportunity to use autologous stem cell transplants as regenerative therapies [1]. Fat is an active and dynamic tissue composed of several different cell types, including adipocytes, fibroblasts, smooth muscle cells, endothelial cells, and angiogenic progenitor cells called pre-adipocytes [2–5]. Stem cells isolated from liposapires have demonstrated broad in vitro adipogenic, chondrogenic, osteogenic, and myogenic lineage commitment [6,7] as well as the ability to differentiate into pancreatic cells, hepatocytes, and neurogenic cells [8–10]. Cytometric analysis of adipose-derived stem cells (ASCs) has shown that these cells do not express CD31 and CD45, but express CD34, CD105, and the mesenchymal stem cell marker CD90 [11,12]. The differentiation potential of ASCs is similar to that of other mesenchymal stem cells, and their yield upon isolation and proliferative rate in culture are higher than those of bone marrow–derived stem cells [13–15]. Treatment with ASCs is considered essential for tissue regeneration owing to their chemotactic, paracrine, and immunomodulatory activities and in situ differentiation [16–19]. Adipose-derived stem cells secrete a cytokine profile that is angiogenic, immunosuppressive, and antioxidative [20]. 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 ability to induce tissue neovascularization [21]. Further, adipose-derived stem cells are immune-privileged because of a lack of human leukocyte antigen–DR expression and suppression of proliferation of activated allogenic lymphocytes [22,23]. Adipose-derived stem cells also inhibit the production of inflammatory cytokines and stimulate the production of anti-inflammatory cytokines [20,24–26]. ASCs are part of the stromal vascular fraction (SVF) of adipose tissue, along with a heterogeneous population of many other cell types including preadipocytes, endothelial cells, pericytes, haematopoietic-lineage cells, and fibroblasts [27]. The regenerative features of the SVF are attributed to its paracrine effects. SVF cells secrete vascular endothelial growth factor, hepatocyte growth factor, and transforming growth factor-β in the presence of stimuli such as hypoxia and other growth factors [28] and strongly influence the differentiation of stem cells, promoting angiogenesis and wound healing and potentially aiding new tissue growth and development [29,30]. Owing to these properties and the ease of harvesting in large amounts with minimal donor-site morbidity, ASCs are particularly promising for regenerative therapies [13,15,19,30]. A variety of tissues and organs...
engineered using ASCs have been described [31]. In vitro studies rapidly progressed to in vivo experiments, wherein ASCs were tested with or without appropriate scaffolds in order to assess their capability to effectively regenerate and repair tissues or organs [31]. However, there have been very few clinical reports of the use of ASCs in cell therapy in humans. Studies presented in literature mostly used basic-research-derived protocols and/or other non-standardised protocols to isolate ASCs for clinical application, which led to inconsistencies in the results [32–45]. Hence, a standardised ASC isolation method for clinical purposes is necessary, which optimizes and unifies process schedule and isolation procedure, as well as whole tissue manipulation.

2. Isolation of ASCs

Initially, fragments of human tissue were minced by hand; however, with the development of liposuction surgery, this procedure has been simplified. During tumescent liposuction, plastic surgeons infuse the subcutaneous tissues with a saline solution containing anesthetic and/or epinephrine via a cannula and then remove both the liquid and tissue under suction [46,47]. The procedure generates fine tissue fragments whose size depends on the dimensions of the cannula. Independent studies have shown that liposuction aspiration alone does not significantly alter the viability of isolated SVF cells [46,48–50]. Adherent stromal cells with characteristics of adipocyte progenitors are present in the liposuction aspiration fluid as well as in SVF derived from tissue fragment digests [46,51]. However, when ultrasound-assisted liposuction is performed, the number of cells recovered from the tissue digests is reduced, as is their proliferative capacity [46,50]. The recovery of ASCs can be improved further by manipulating the centrifugation speed [46,52]. Investigators have achieved optimal cell recovery by using a centrifugation speed of 1200 g. In 2001, Zuk et al. [53,54] first isolated ASCs from adipose tissue after a liposuction procedure, by using existing enzymatic strategies. Since then, interest in ASCs has grown dramatically and several groups working independently have developed procedures to isolate and characterize ASCs. Zuk et al. [54] described a protocol wherein the freshly harvested lipoaspirate was washed with sterile phosphate buffered solution to remove blood cells, saline, and local anesthetics. It was then enzymatically digested using 0.075% collagenase type I, which was subsequently inactivated by the addition of an equal volume of Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Finally, red blood cell lysis was performed and the high-density ASC pellet separated from the infranatant by centrifugation. In 2016, Raposo et al. [11,13] described a method that was specifically designed for clinical application, which appeared easy, safe, and fast (80 min), allowing collection of a ready-to-use ASC pellet. After a conventional liposuction, the harvested fat tissue (100 ml) was subjected to a first centrifugation (1600 RPM x 6 min), yielding about 50 ml of high quality concentrated adipose tissue. This was abruptly mixed with 50 ml collagenase digestion solution (Collagenase NB 6 GMP Grade 17458; Serva GmbH, Heidelberg, Germany), previously diluted with sterile phosphate-buffered saline (PBS) as follows: 1 g of collagenase was suspended in 10 ml PBS, and 1 ml of the obtained solution was further diluted with 49 ml of PBS. The solution obtained (lipoaspirate + collagenase digestion solution) was then incubated for 30 min at 37 °C in a shaker-incubator (Geltltabor; Medi-khan) and centrifuged at 200 relative centrifuge force for 4 min. Subsequently, the 10 ml of SVF obtained was washed 2 times with 45 ml saline solution. After each wash, the syringes containing SVF were centrifuged at 200 relative centrifuge force for 4 min. The cellular pellet obtained at the bottom of the syringe was ready for use, vehiculatied by 5 ml of saline solution. Several alternative isolation methods have been proposed, which avoid enzymatic digestion completely. Yoshimura et al. [51] found that a significant number of ASCs could be isolated from “liposuction aspirate fluid”, and although cell numbers were less than those obtained with enzymatic digestion, they were sufficient for clinical use without cell expansion [13]. Raposo et al. [12] also described an effective alternative mechanical procedure in which the isolation process was performed using a vibrating shaker (Multi Reax; Heidolph, Schwabach, Germany) and a centrifuge (MPW 223; Johnson & Johnson Medical, New Brunswick, N.J.), both placed in a laminar air flow bench (1200 FLO; FIMS, Concorezzo, Italy). After liposuction, the harvested fat tissue (80 ml) was collected in eight 10-ml plastic test tubes, positioned in the vibrating shaker at 6000 vibrations/minute for 6 min, and immediately centrifuged at 1600 rpm for 6 min. Subsequently, under the same laminar flow cabinet, the pellet at the bottom of each tube was collected by means of an automated pipetting system (Rota-Filler 5000; Heathrow Scientific, Nottingham, United Kingdom) and poured into a 10-ml Luer-Lock syringe. The entire isolation process lasted approximately 15 min [13].

3. Culture and expansion of ASCs

ASCs obtained from humans and other species exhibit a fibroblast-like appearance and the potential to differentiate into adipogenic, osteogenic, chondrogenic, myogenic, and neurogenic lineages under appropriate culture conditions [55]. Generally, ASCs have a cell doubling time of 2–4 days, depending on donor age, tissue type (white or brown adipose tissue) and location (subcutaneous or visceral), type of surgical procedure, culture conditions, plating density, and media formulations [56,57]. The proliferation of ASCs can be stimulated using several exogenous supplements including fibroblast growth factor 2 (FGF-2) via the FGF receptor 2 [58], sphingosylphosphorylcholine via the activation of c-jun N-terminal kinase (JNK; [59]), platelet derived growth factor via the activation of JNK [60], and oncostatin M via activation of the microtubule associated protein kinase (MEK) extracellular signal regulated kinase (ERK) and JAK3STAT1 pathway [61]. On the other hand, Rubio et al. have shown that human ASCs undergo malignant transformation with prolonged passages for more than 4 months [62]. Thus, care must be taken in the manipulation and culture of ASCs. Moreover, freshly isolated ASCs might be safer and more practical for clinical use than cultured ASCs.

4. Multilineage differentiation of ASCs

The multipotency of ASCs has generated interest in their potential therapeutic value in regenerative medicine. Differentiation of these cells can be directed by the addition of specific cocktails of chemical inducers or cytokines. The differentiation efficiency is patient-dependent. The age of the donor can be a factor; some studies suggest that the differentiation capacity is higher in cultures from younger subjects compared to those from older people [63]. Human and mouse ASCs can differentiate into adipocytes. Adipocyte regeneration is useful for the reconstruction of breast tissue after surgery for breast cancer and asymmetry as well as soft tissue and subdermal defects due to trauma, surgery, or burn injury [64–67]. When the cells reach 80–90% confluence, adipogenic differentiation of the preadipocytes can be induced [63]. ASCs cultured in media containing IBMX, insulin, dexamethasone, mesenchymal cell growth supplement (MCCS), and 1-glutamine for 2 weeks became lipid-retaining cells that were stained by oil red [67]. Osteogenesis can be induced using culture medium supplemented with 1 nM dexamethasone, 2 mM β-glycerophosphate, and 50 μM ascorbate-2-phosphate. The cells are induced in this medium for approximately 14 days and the osteogenic medium is replaced every 2–3 days. Mineralization is assessed by staining the cells with 40 mM Alizarin Red (pH 4.1) after fixation in 10% formalin [63]. For chondrogenesis [68], cell pellets are cultured in chondrogenic differentiation medium, which consists of high-glucose DMEM supplemented with 500 ng/ml bone morphogenetic protein-6, 10 ng/ml TGF-β3, 107 M dexamethasone, 50 μg/ml ascorbate 2-phosphate, 40 ng/ml proline, 100 μg/ml pyruvate and 50 mg/ml ITS + premix (Becton Dickinson: 6.25 μg/ml insulin, 6.25 μg/ml transferrin, 6.25 ng/ml selenious acid, 1.25 mg/ml bovine serum albumin, 5.35 mg/ml linoleic acid). The medium is replaced
every 2–3 days for 21 days. The pellets are then fixed in formalin, embedded in paraffin and sectioned. The sections can then be stained with Toluidine Blue [63,68]. In 2009, Coradegni et al. [8] performed hepatic induction of ASCs over a period of 5 weeks. After ASCs reached 80% confluency, two different hepatic differentiation procedures were performed in a humidified atmosphere at 37 °C and 5% CO2. The first differentiation procedure, as described by Talens-Visconti et al. [69], involved the culture of ASCs for 48 h in DMEM serum free with 20 ng/ml epidermal growth factor (EGF) and 10 ng/ml bFGF (Invitrogen Life Technologies, Carlsbad, Calif., USA). In the second differentiation procedure, which was that reported by Seo et al. [70] with minor modification, ASCs were incubated for 2 weeks in DMEM-LG (low glucose) with 5% FBS, 1 mM dexamethasone, 10−4 M ascorbic acid 2-phosphate, 10 ng/ml EGF, 5 ng/ml insulin, 5 ng/ml transferrin, and 5 ng/ml sodium selenite. Hepatogenic differentiation was induced by culturing ASCs for 5 weeks in the same medium without serum, with 0.1% DMSO, 10 ng/ml HGF, and 10 ng/ml OSM (medium B). All culture media was changed twice a week and all cultures were observed daily through microscopy to estimate changes in cell morphology. Safford et al. [71] reported neuronal differentiation of ASCs, and showed that murine and human ASCs differentiate into neuronal tissue when cultured with valproic acid, forskolin, hydrocortisone, and insulin. In 2010, Scaranotti et al. [72] described a neurogenic differentiation procedure wherein cellular samples were grown in neurogenic medium consisting of DMEM with 10% human serum, 2 mM glucose, penicillin (100 U/ml)-streptomycin (50 g/ml), amphotericin B (2.5 g/ml), 5 g/ml insulin, 200 M indomethacin, and 0.5 mM isobutylmethylxanthine. Control cultures were simultaneously maintained under standard culture conditions. Culture medium was replaced with fresh medium twice a week. To assess the stage of neurogenic development, control cells and induced cells were observed daily under the microscope (Nikon, Eclipse TS100) before specific neural markers were analysed. All differentiation procedures were performed for two weeks. In 2009, Okura et al. [73] demonstrated that human ASCs differentiate into pancreatic islet-like cluster cells by using specific culture conditions in four steps. First, they used Dulbecco’s modified eagle medium (DMEM) (60%), MCDB 201 (40%) containing medium DMEM, human serum, glutamine, penicillin, streptomycin, and indomethacin, and exendin-4 (100 ng/ml) epidermal growth factor (EGF), bFGF (Invitrogen Life Technologies, Carlsbad, Calif., USA). In 2012, Gir et al. [26] reported published and ongoing clinical trials on applications of ASCs. In plastic surgery, ASCs were usually used in the form of autologous stromal vascular fraction cells or non-cultured adipose-derived stem cells. According to literature, adipose-derived stem cells have been used or studied in three main areas: soft tissue augmentation, wound healing, and tissue engineering [3,75–78]. As regards clinical application of ASCs for soft-tissue augmentation, Yoshimura et al. [79–81] 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 [79]. In 2011, Tiriyaki et al. [82] and Kamakura et al. [83] published two additional non-controlled trials using cell-assisted lipotransfer for breast augmentation. Kim et al. [84] published the results of a clinical trial involving 31 patients treated for depressed facial scars with adipose-derived stem cells that were differentiated into mature adipocytes; however, there was no comparative control group. Akita et al. [85] reported on a case of one patient with an intractable wound in the sacro-coccygeal region secondary to radiation therapy. 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. In 2007, Rigotti et al. [86] published a study on 20 patients undergoing therapy with centrifuged liposapirate for side effects of radiation treatment, with severe symptoms and irreversible functional damage from radiation wounds. An improvement in tissue wound healing was observed, and the authors postulated that the liposapirate was rich in native adipose-derived stem cells that contributed to the observed effect. Stillaert et al. [87] described the use of ASCs in tissue engineering in 2008. They inserted subcutaneously implanted hyaluronic acid scaffolds seeded with adipose-derived stem cells into 12 volunteers. No new adipose tissue formation was observed, which was attributed to a deficient angiogenic response that was insufficient to sustain long-term adipose-derived stem cell viability without adverse effects. In 2011, Kim et al. [84] reported on various clinical trials on the use of ASCs in plastic surgery: In a phase I study of autologous adipose-derived stem cell transplantation in Brazil in patients with lipodystrophy, lipoinjection enriched with adipose-derived stem cells is being performed, and five subjects were enrolled. The primary outcome measure was volume improvement in the transplanted area. In a phase II study in Korea on the use of ASCs in treating Romberg disease, five subjects were enrolled. The primary outcome measure was the volume change in the fatty layer using three-dimensional image analysis. In Korea, phase II and III studies on the safety and efficacy of autologous cultured adipose-derived stem cells were performed in patients with depressed scars, and 36 subjects were enrolled to receive cultured ASCs differentiated into mature adipocytes (AdipoCell). The primary outcome measures assessed were scar improvement and safety. A phase IV study conducted in Belgium, Italy, Spain, and UK examined the use of autologous fat enhanced with ASCs for

5. Clinical applications of adipose-derived stem cells

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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. To date, adipose-derived stem cell therapy has been used in a wide variety of specialized applications. In some cases, allogenic or autologous ASCs were used, while SVF or cultured ASCs were used in others. Some studies included additive treatments (e.g., bone marrow, growth factor, fibrin glue). The ASC doses were variable, as was the number of ASCs per dose. Adipose-derived stem cells were administered systemically (intravenous infusion) or locally (intraleisional injection) [26]. From 2006 to 2011, Fang et al. [88] used allogenic adipose-derived stem cells in the treatment of hematologic and immunologic disorders such as graft versus-host disease, idiopathic thrombocytopenic purpura, or pure red cell aplasia [88–93]. In each case, patients received intravenous infusions 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 cases of idiopathic thrombocytopenic purpura. In 2008, Trivedi et al. [94] treated five patients with diabetes mellitus type 1 by using allogenic adipose-derived stem cells cultured and differentiated into insulin-making mesenchymal stem cells mixed with unfractionated autologous cultures of bone marrow. No adverse effects were reported, and all subjects were reported to be healthier and gaining weight, with improvements in biological markers. In 2010, Vanikar et al. [95] reported the results obtained from 11 patients with diabetes using the same treatment. No adverse events were reported, and a gradual decrease in insulin requirement was noted. From 2003 to 2010, García-Olmo et al. [96] published several articles on phase I and II clinical trials on the treatment of complex perianal or entero-cutaneous fistulas. The trials included patients with digestive fistulas associated or not associated with Crohn disease, treated using autologous adipose-derived stem cells isolated mixed with fibrin glue and injected into the fistulous tract [96–101]. In all the studies, no adverse effects were reported and a significant healing rate was observed in patients who received ASCs. Rheumatoid arthritis treatments were examined in a case report in 2010 by Ichim et al. [102] of a 67-year-old woman. The patient was treated by intravenous infusions of autologous stromal vascular fraction cells isolated from a liposuction procedure. No side effects were observed, and the patient reported a considerable resolution of her joint pain and stiffness, with a decrease in rheumatoid factor. In 2009, Riordan et al. [103] reported the treatment of three patients with multiple sclerosis 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. No adverse effects were documented, and all patients reported significant improvement of their symptoms within 3 months. In 2008, Alvarez et al. [104] reported the case of a 67-year-old man suffering from lung cancer complicated with tracheal mediastinal fistula, treated by autologous adipose-derived stem cells mixed with fibrin glue, injected into the fistula during bronchoscopy. In 2004, Lendecel et al. [105] published the case of a 7-year-old girl suffering from widespread calvarial defects after severe head injury. The patient was treated with a combination of cancellous bone grafts from the ilium and autologous stromal vascular fraction cells obtained from adipose tissue. Three months postoperatively, the computed tomographic scan showed marked ossification in the defect areas. In 2009, Mesimaki et al. [106] reported the case of a 65-year-old patient who underwent a hemi maxillectomy because of a large keratocyst. Reconstruction was performed using a preformed titanium cage filled with autologous cultured adipose-derived stem cells, combined with synthetic bioreabsorbable beta-tricalcium phosphate granules. No adverse effects were reported, and bone regeneration was observed by biopsy. In 2010, Taylor [107] 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, periostel grafts, and autologous fresh adipose-derived stem cells. 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. In 2011, Pak [108] reported two cases of patients suffering from osteonecrosis of the femoral head and two cases of patients suffering from knee osteoarthritis. All the patients were treated with a combination of percutaneously injected autologous adipose-derived stem cells, hyaluronic acid, platelet-rich plasma, and calcium chloride. Magnetic resonance imaging scans showed 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 height of meniscus cartilage. In 2010, Yamamoto et al. [109] reported two cases of patients with stress urinary incontinence, 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.) injected (mixed with adipose tissue) in the external urethral sphincter under endoscopic vision. No adverse effects were observed. Urinary incontinence improved progressively after 2 weeks, up to 12 weeks. In 2011, Ra et al. [110] published a study of eight patients suffering from spinal cord injury who were treated with intravenous infusions of autologous adipose-derived stem cells. The authors observed no serious adverse effects. At 12 weeks, motor function was improved in four patients (Table 2).

6. US and European ASCs regulation

In the United States, adipose-derived stem cells fall in the category 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 [26,111]. 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 aim to prevent the introduction, transmission, or spread of communicable disease by human cells, tissues, or cellular and tissue–based products [26,112]. 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 [26,111]. This is true if it meets all the following criteria (Part 1271.10): (a) The human cell, tissue, or cellular and tissue–based product is minimally manipulated. (b) The human cell, tissue, or cellular and tissue–based product is intended for homologous use only. (c) 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. (d) 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 non-tissue 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 proven 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

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Table 2
Clinical applications of adipose-derived stem cells.

| Clinical applications                                                                 | Patients | References |
|---------------------------------------------------------------------------------------|----------|------------|
| Cell-assisted lipotransfer (facial lipoinfusia), cosmetic breast augmentation, or immediate breast augmentation after breast implant removal | 20       | [79-81]    |
| Cell-assisted lipotransfer for breast augmentation                                      | 29       | [82]       |
| Sacro-coccygeal region wound secondary to radiation therapy                            | 1        | [85]       |
| Wounds secondary to radiation treatment                                                | 20       | [86]       |
| Tissue engineering (subcutaneous hyaluronic acid scaffolds seeded with adipose-derived stem cells) | 12       | [87]       |
| Lipodystrophy (Phase I study, Brazil)                                                  | 5        | [84]       |
| Romberg disease (phase II and III studies, Korea)                                       | 5        | [84]       |
| Depressed scars treated by cultured ASCs (phase II and III, Korea)                     | 36       | [84]       |
| Breast reconstruction after lumpectomy with autologous fat enhanced with ASCs (phase IV study, Belgium, Italy, Spain, UK) | 71       | [84]       |
| Intravenous infusion of allogenic ASCs to treat hematologic and immunologic disorders (graft versus-host disease, idiopathic thrombocytopenic purpura, or pure red cell aplasia) | 14       | [88-93]    |
| Diabetes mellitus type I treated by infusion of allogenic cultured ASCs differentiated into insulin-making mesenchymal stem cells and mixed with unfractionated autologous cultures of bone marrow | 5        | [94]       |
| Perianal or enter-cutaneous fistulas associated or not associated with Crohn disease treated using ASCs mixed with fibrin glue and injected into the fistulous tract | 11       | [95]       |
| Rheumatoid arthritis treatments treated by intravenous infusions SVF                   | 1        | [102]      |
| Multiple sclerosis treated by intravenous infusions of SVF with multiple intrathecal and intravenous infusions of allogenic CD34 and mesenchymal stem cells | 3        | [103]      |
| Lung cancer complicated with tracheal mediastinal fistula, treated by ASCs mixed with fibrin glue, injected into the fistula during bronchoscopy | 1        | [104]      |
| Widespread cutaneous defects after severe head injury due to bone graft and injection of SVF | 1        | [105]      |
| Post hemi maxilloctomy treated by titanium cage filled with ASCs combined with synthetic bioreasorbable beta-tricalcium phosphate granules | 1        | [106]      |
| Treacher Collins syndrome treated by bone allograft, bone morphogenetic protein-2, periosteal grafts, and ASCs | 1        | [107]      |
| Osteonecrosis of the femoral head treated with injection of ASCs, hyaluronic acid, platelet-rich plasma, and calcium chloride | 2        | [108]      |
| Knee osteoarthritides treated with injection of ASCs, hyaluronic acid, platelet-rich plasma, and calcium chloride | 2        | [108]      |
| Stress urinary incontinence treated with ASCs injected (mixed with adipose tissue) in the external urethral sphincter | 2        | [109]      |
| Spinal cord injury treated with ASCs intravenous infusions                              | 8        | [110]      |

Table 3
Comparison between United States and European regulation.

| Definition of ASCs | United States | Europe |
|--------------------|---------------|--------|
| Minimally manipulated ASCs | | |
| Human cells, tissues, or cellular and tissue-based products intended for implantation, infusion, or transfer into a human recipient | | |
| Homologous use | | Minimally manipulated (non-cultured, isolated mechanically) |
| Not in combination with other articles | | Subcutaneous tissue recipient site only |
| Does not have a systemic effect and is not dependent on metabolic activity of living cells for its primary function otherwise it has to be for autologous use. | | |
| Regulation | Current Good Tissue Practice (Code of Federal Regulations, Title 21, Part 1271) | None |
| More than minimally manipulated ASCs | Advanced therapy: |
| More than minimal manipulation (e.g., ex vivo expansion) | | |
| Combination with non-tissue components | | |
| Transduction | Cells that underwent substantial manipulation (e.g., ex vivo expansion, non-mechanically isolated) |
| Heterologous use | | |
| Having a systemic effect and is dependent on metabolic activity of living cells for its primary function and it is not autologous use. | | |
| Gene therapy medicinal product, somatic cell therapy medicinal product, or tissue engineered product. | | |
| Regulation | Section 351 of the Public Health System Act | Regulation and the Directive 2001/83/EC Annex I Part IV30 definition of (ATMPs) |
| Additional | U.S. Food and Drug Administration regulations (Title 21, Code of Federal Regulations, Part 312) | Article 17 of Regulation (EC) No 1394/2007 (the Advanced Therapy Medicinal Products (ATMPs) Regulation) |
| U.S. Food and Drug Administration | | Committee for Advanced Therapies |

ASCs: Adipose-derived Stem Cells; EC: European Commission.

in effect as specified by U.S. Food and Drug Administration regulations (Title 21, Code of Federal Regulations, Part 312; [113]). Automated devices for separating adipose stem cells are regulated as class III medical devices by the U.S. Food and Drug Administration [26,114]. 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 [26,115]. In Europe, the use of ASCs is regulated differently if they are considered advance therapy (ATMPs) or less. The ATMP Regulation and the Directive 2001/83/EC Annex I Part IV30 provide precise legal definitions for ATMPs [116]. After the implementation of Article 17 of Regulation (EC) No 1394/2007 (the Advanced Therapy Medicinal Products (ATMPs) Regulation), applicants have access to an optional procedure, which is the CAT (Committee for Advanced Therapies) scientific recommendation for the classification of ATMPs [117]. ATMP classification is based on the evaluation of whether a given product fulfills one of
the definitions of gene therapy medicinal product (GTMP), somatic cell therapy medicinal product (sCTMP), or tissue engineered product (TEP). The Committee is responsible for assessing the quality, safety, and efficacy of advanced therapy medicines, including medicines classified as gene therapy, somatic cell therapy, or tissue-engineered products. It is underpinned by the ATMP regulation, which enables the EMA, in close collaboration with the European Commission, to determine whether a given product meets the scientific criteria, which define ATMPs. The ATMP classification procedure has been established in order to address borderline cases where classification of a product based on genes, cells, or tissues is not clear. The CAT issues scientific recommendations determining whether the referred product falls within the definition of an ATMP in the European Union. In 2015, the Committee for Advanced Therapies (CAT) stated that the use of collagenase for separation of cells from extracellular matrix of tissue is considered a substantial manipulation and thus an advanced therapy [118]. Therefore, enzymatic digestion would be assessed on a case-by-case basis depending on the nature of the tissue to be digested, and deviation is be possible when scientific evidence is provided. Cell culture leading to expansion is considered an advanced therapy because of substantial manipulation. Furthermore, ASCs intended for use in other tissues except for adipose subcutaneous tissue are always considered an advanced therapy medicinal product. According to these considerations, ASCs should be non-cultured, isolated mechanically, and used only in the subcutaneous tissue [119] (Table 3).

7. Discussion

The clinical applications of ASCs are numerous and varied; the ease of cell harvest and high yield with minimal donor-site morbidity makes adipose tissue an ideal stem cell source. Additionally, the multi-lineage potential of these cells presents significant opportunities for use in the field of tissue engineering, with studies successfully demonstrating their ability to produce a wide range of tissue types [120]. 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 experimental studies with standardised 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 [26]. In Europe and the United States, the current regulatory guidelines allow the use of ASCs only in limited cases, so that most of the clinical applications described over the last decade are prohibited. As previously reported, because of the rigors of safe, reproducible, quality-controlled adipose-derived stem cell production as required by the U.S. Food and Drug Administration, the use of adipose-derived stem cells in clinical trials or for clinical applications is very rare in the United States. In Europe, the regulations are different depending on whether the therapy is considered advance therapy or not. After the implementation of Article 17 of Regulation (EC) No 1394/2007 (the Advanced Therapy Medicinal Products (ATMPs) Regulation), the CAT (Committee for Advanced Therapies) issues scientific recommendations for the classification of ATMPs. Gene therapy medicinal products (GTMPs), somatic cell therapy medicinal products (sCTMPs), and tissue-engineered products (TEPs) are considered ATMPs. However, ASCs therapy does not always fall into this classification. Cell populations derived by mechanical purification of tissue are not considered a sCTMP. Examples include cryopreserved ASCs or regenerative cells and suspensions of viable, adult, autologous, unexpanded, and uncultured regenerative cells of stromal vascular fraction from subcutaneous adipose tissue (EMA/500724/2012, EMA/129056/2013). In a recent scientific recommendation [119] on classification of advanced therapy medicinal products, the EMA evaluated the clinical use of adult autologous ASCs for subcutaneous administration. In this recommendation, ASCs were described as suspension of viable, adult, autologous, unexpanded, and uncultured regenerative cells of the stromal vascular fraction of subcutaneous adipose tissue. Therefore, ASCs that are non-cultured, isolated mechanically, and used locally for regeneration of subcutaneous tissue (homologous tissue) are not classified as ATMPs. To date, European clinical trials involving the use of ASCs for regeneration of non-homologous tissue (e.g., for digestive disease, autoimmune disease, cardiovascular disease, skeletal regeneration, and neurologic disorder) should be considered off-label. Further, clinical trials involving the use of collagenase that caused change of structural and functional properties of ASCs or the use of expanded or cultured ASCs should be considered off-label.

8. Conclusion

In Europe, ASCs intended for use in tissues other than adipose subcutaneous tissue are always considered an advanced therapy medicinal product. The use of collagenase for separation of cells from extracellular matrix of tissue is considered a substantial manipulation; however, enzymatic digestion could be assessed on a case-by-case basis by CAT and would depend on the nature of the tissue to be digested. Deviations from the regulations are therefore possible when scientific evidence is provided. According to these considerations, ASCs should be non-cultured, isolated mechanically, and used only in the subcutaneous tissue.

Ethical approval

There was no need for Ethical Approval.

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Author contribution

Prof. Raposio Edoardo, study design.
Dr. Simonacci Francesco, writing.
Dr. Nicolò Bertozzi, writing and final revision of the manuscript.

Conflicts of interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

Guarantor

Prof. Raposio Edoardo.
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