Adipose-Derived Stem Cells: Isolation and Utilization within Regenerative Medicine and Cosmetic Procedures

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Received Date: 12-02-2022; Accepted Date: 14-03-2022; Published Date: 21-03-2022

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Abstract

Adipose-derived stem cells are a promising tool for the future of reconstructive surgeries, regenerative therapies and cosmetic procedures. Their ability to be safely, efficiently and cost-effectively isolated from extracted subcutaneous fat tissue makes them a favorable option for plastic surgeons, who often routinely perform liposuction procedures that provide adequate samples for stromal vascular fraction isolation. The differentiation potential of adipose-derived stem cells is vast and limited only in a developmental potential when compared to embryonic stem cells. Their utilization within clinical trials can be seen in procedures ranging from soft tissue and bony reconstruction, wound and burn healing, peripheral nerve regeneration and cosmetic procedures like skin rejuvenation and breast augmentations. The results from these trials provide evidence that supports future use of these stem cells within these fields of medicine.

Keywords

Adipose-Derived Stem Cells; Harvesting Isolation; Lipoaspirate; Stromal Vascular Fraction; Regenerative Medicine; Cell-Assisted Lipotransfer; Plastic Surgery
Abbreviations


Introduction

Stem cells provide an abundance of potential for regenerative medicine and cosmetic surgeries on account of their unique ability to divide and differentiate into multiple cell lineage pathways [1]. For stem cells to be utilized for medical procedures, they must be

1. Found in prolific quantities- numbers ranging from millions to billions of cells
2. Able to be harvested through a minimally invasive procedure
3. Able to differentiate into multiple cell lineage pathways in a reproducible manner
4. The transplant of cells to the host, either autologous or allogenic, must be effective and safe [2]

Embryonic Stem Cells (ESCs) provide the most straightforward solution to this criterion because of their high regenerative capacity. Their pluripotent capabilities allow ESCs to differentiate into cells of mesodermal, endodermal and ectodermal origins; creating the diverse ability to generate any cell type needed [3]. Successful ESC isolation has led to conclusive research on the regenerative properties of these stem cells, but ethical controversy encompasses the origin and method of isolation of ESCs and concerns of tumorigenicity and immunocompatibility have ultimately led to limited clinical research [3]. Postnatal Adult Stem cells (AS) function as multipotent cells rather than pluripotent. They have the potential to differentiate into almost any cell type needed but are limited in their developmental potential compared to ESCs [3]. Mesenchymal Stem Cells (MSCs) are a type of AS that are isolated from virtually any tissue in the body, such as adipose tissue, trabecular bone, skin, skeletal muscle, pericytes, umbilical cord blood, periosteum, peripheral blood, synovial membrane, dermis, dental pulp, periodontal ligament and tumors [1]. Although present in these tissues, only a select few provide the abundant quantity needed to meet the requirements for harvesting and medicinal utilization [1].
Adipose-derived Stem Cells (ASCs) have proven to be the most promising source of stem cells due to their plenteous supply within the human body and their ability to be harvested with minimal adverse effects to the donor [1,4,5]. Studies have shown that about 2-3 x 10^8 ASCs can be harvested from 300 ml of adipose tissue which yields 100 to 1000 times more stem cells than those harvested from the same volume of bone marrow [4]. Common extraction locations include the abdomen, thigh and arm in which cells are isolated from the subcutaneous adipose tissue [1]. ASCs have been observed to have immunomodulatory properties, such as migrating to the site of infection and stimulating tissue repair [6]. ASCs do not express MHC-II protein tags and low levels of MHC-I proteins, which allows for high success during transplantation due to a low recipient rejection rate [6]. Minimum criteria for identification of ASCs as stated by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT) includes

1. Adherence to plastic surfaces
2. Expression of CD73, CD90, CD105 and lack of expression of CD11b, CD14, CD19, CD79a, CD45, CD34 and HLA-DR

The method used to extract fat tissue has been shown to greatly affect the viability, yield, proliferative index and stemness of harvested ASCs. When comparing results from three methods, surgical resection, Power-Assisted Liposuction (PAL) and Laser-Assisted Liposuction (LAL), it was found that ASCs harvested using PAL had the highest proliferation rate and the slowest rate of senescence [6].

Advances in plastic and reconstructive surgeries have allowed for the utilization of stem cells to aid surgical procedures. The ability of stem cells to multiply and differentiate into numerous cell lineages makes them an attractive solution to many regenerative tissue operations as well as cosmetic surgeries [3]. Plastic surgeons are put in a unique position because of how often they perform cosmetic liposuction and breast reduction procedures. With consent from the patient, the extracted subcutaneous fat provides the perfect sample to isolate ASCs for use in other procedures [7]. Clinical studies have shown that ASCs aid in a variety of procedures ranging from soft tissue and bony reconstruction, wound and burn healing, peripheral nerve regeneration and cosmetic surgeries like skin rejuvenation and breast augmentations [1-4,7-10]. In this review, we aim to focus on the isolation techniques specific to ASCs and clinical applications throughout regenerative and cosmetic surgeries.

**Isolation of ASCs**

To date, there is no standardized procedure for the isolation of ASCs from lipoaspirate for use in clinical applications. The most common method of ASC isolation is through enzymatic digestion to obtain a pellet of Stromal Vascular Fraction (SVF). The SVF pellet contains a mixture of endothelial cells, pericytes, endothelial progenitor cells, smooth muscle cells, erythrocytes, leukocytes and most importantly ASCs [11]. Research has shown the most
An effective method of extraction of SVF is the Mechanical and Enzymatic (ME) procedure and studies have resulted in a greater number of viable isolated ASCs using the ME method [8,12]. From a 100 ml extract of lipoaspirate, a mean value of 6.25 x 105 ASCs can be isolated from a solely Mechanical (MC) procedure in comparison to 9.06 x 105 ASCs from an ME procedure [12]. Guidelines set by the Food and Drug Administration (FDA) state that lipoaspirate and autologous fat transfer is classified under the human cells, tissues, or cellular or tissue-based products (HCT / P) regulations, which state that the tissue must undergo minimal manipulation and be used for a homologous procedure [6,13,14]. Isolated lipoaspirate meets these standards, but issues arise when enzymes, like collagenase, are introduced to digest the adipose tissue to create the ASC-rich SVF [13,15]. The presence of the enzymes and highly concentrated levels of ASCs in the separated fraction classifies them under different regulations that allow the clinical use of ME isolation under strict supervision [14,15]. In the United States, clinical trials involving SVF must be approved as Investigational New Drug Application and overseen by the FDA and due to these strict guidelines most clinical applications involving SVF are seen in countries outside of the United States [14]. To combat this problem, some scientists are trying to utilize strictly MC isolation procedures to create a more natural but equally efficient way to isolate SVF [6,16]. These methods aim to utilize physical forces, like centrifugation and vortexing, to separate the adipose matrix in harvested lipoaspirate; however, no consensus has been reached [16]. Although it is debated that enzymatic digestion to obtain isolated SVF may be dangerous, no conclusive evidence has been presented to prove such a claim [15]. Both methods are being explored and currently the ME method is still preferred due to its efficiency and because of recent technological advances in machines that handle the digestion and isolation process for the doctor or researcher [13].

ME Method of Isolation

A proposed method for clinical use of ME isolation aims to isolate SVF from harvested lipoaspirate and then digest the solution using enzymes to release ASCs. A completely closed circuit is necessary to avoid contamination from the open air and tubes can be used to transfer solution from syringe to syringe [17]. Researchers have modified the most widely accepted method of isolation to make it more practical for clinical applications [18]. This ME method isolates SVF from harvested lipoaspirate which takes about 80 minutes from the time the tissue is harvested to the time the SVF is ready to be injected into the new host (Fig. 1)[17]. To do so, 100 ml of lipoaspirate harvested from a healthy adult patient is centrifuged for 6 minutes at 1600 Rounds Per Minute (RPM). This yields around 50 ml of highly concentrated adipose tissue that is mixed with 50 ml of a previously prepared collagenase digestion solution- 1 g of collagenase is suspended in 10 ml of Phosphate-Buffered Saline (PBS). One ml of the mixed solution is then added to a new syringe and 49 ml of PBS is added to dilute. The lipoaspirate is incubated with the digestion solution at 37°C for 30 minutes in a shaker-incubator. The solution is then centrifuged for 4 minutes at 200 Relative Centrifugal Force (RCF), resulting in only 10 ml of SVF remaining. The SVF is washed with a 45 ml saline solution and the syringe containing the SVF is centrifuged for 4 minutes at 200 RCF. This step is repeated to obtain a
 usable pellet of SVF that can be mixed with 5 ml of saline solution to resuspend the pellet for injection.

Figure 1: Visual of Raposio, et al. ASC isolation technique [17]. 1: Conventional liposuction is performed to extract lipoaspirate, 2: Harvested fat tissue is centrifuged at 1600 RPM for 6 minutes, 3: Highly concentrated adipose tissue is isolated from centrifuged syringe, 4: Adipose tissue is combined with collagenase digestion solution (1g of collagenase NB 6 GMP Grade to 10ml PBS), 5: Mixed solution is incubated at 37ºC for 30 minutes in a shaker-incubator, 6: Digested tissue is transferred into a clean syringe, 7: Syringe is centrifuged for 4 minutes at 200 RCF, washed with a saline solution and centrifuged at 200 RCF for 4 minutes, the wash and centrifuge are repeated once, 8: ASC rich cellular pellet is extracted and mixed with 5 ml of saline solution, 9: ASC rich solution is injected into the area of interest.

Induced Differentiation of ASCs

Most often, ASCs are merely utilized as is and are not differentiated prior to injection due to the cells commonly being used in lipotransfer procedures. In the case of nerve regeneration or bone reconstruction, it has been shown to be beneficial to differentiate the ASCs in-vitro prior to use [9,19]. This can be done through media-induced differentiation in which ASCs are plated on a specific media that prompts lineage-specific induction factors to yield a specific cell type other than an adipocyte [11]. ASCs have the ability to differentiate into osteoblasts, articular and tracheal chondrocytes, nucleus pulposus-like cells, cardiomyocytes, skeletal myocytes,
vascular and visceral smooth muscle cells, endothelial cells and dermal fibroblasts [18,20,21]. They have also been shown to transdifferentiate to ectodermal cells such as keratinocytes, neurons, Schwann cells, retinal pigment epithelial cells and corneal epithelial cells as well as cells of endodermal origin that include hepatocytes and pancreatic islet cells [20,21]. In-vitro, ASC differentiation has been heavily studied and shows promising results but there is no current consensus on the effectiveness or viability of in-vivo differentiation.

**Cell-assisted Lipotransfer**

Extracted lipoaspirate naturally contains ASCs but the quantity of stem cells is too little to be considered a cell-enriched graft. Cell-Assisted Lipotransfer (CAL) is a method of lipoinjection that isolates ASC-rich SVF from harvested lipoaspirate and recombines it with ASC-poor aspirated fat to produce an ASC-rich fat to be used for injection. This method provides a promising solution to the problems of partial necrosis and graft unpredictability by promoting adipocyte survival [14,22]. Harvested lipoaspirate is divided into two samples of equal volume (Fig. 2). Half is digested to extract the SVF, using the isolation techniques described above and the other half undergoes a wash to clean the tissue of any unwanted debris [23]. Studies have shown that using a 1:1 SVF-to-fat ratio yields the most efficient results in terms of greatest mean weight and volume [24]. To maximize survival rate of the grafts it is essential to inject the fat graft in multiple passes into different tissue planes [25]. Years of clinical trials have concluded that the use of CAL is favorable over conventional lipoinjection due to higher efficiency, lower fat resorption and increased fat graft survival [22-24,26]. The success of CAL can be attributed to the ability of ASCs to facilitate adipose tissue regeneration by differentiating into adipocytes, promoting growth of new capillaries in the adipose tissue by inducing vasculogenesis through differentiating into vascular wall and endothelial cells, secreting Hepatocyte Growth Factor (HGF), Insulin-Like Growth Factor-1 (IGF-1) and Vascular Endothelial Growth Factor (VEGF) to promote blood vessel formation and continuously supporting growth and regeneration through division [27].
Clinical Applications

There are many studies involving clinical applications of ASCs within numerous types of regenerative and cosmetic therapies and procedures. The findings of these studies highlight a promising future for the utilization of these stem cells within the fields of plastic and regenerative surgeries.

Bony Reconstruction

ASCs are readily able to differentiate into both osteoblasts and chondroblasts making them a desirable candidate for bony and cartilage regeneration therapies [2]. Promising results were yielded from a clinical trial involving a 7-year-old girl who suffered a severe head injury with multifragment calvarial fractures and presented with widespread calvarial defects [2]. SVF was injected at specific locations and throughout a 3-month period computed tomography scans showed new bone formation [9]. In human trials, this bone forming capability has been shown when ASCs are either seeded alone in β-Tricalcium Phosphate (TCP) granules or combined with autologous bone [9]. Defects within the maxilla and mandible have also been reported to yield favorable results when treated with ASCs [9]. Multistep delayed procedures have used ASCs combined with growth factors in muscle tissues to transplant a microvascular flap. The transplanted muscle is specifically placed to surround ectopic bone and results have shown both functional and aesthetic results in maxilla repair [9]. For mandibular defects, a single-
stage procedure is used to fill the defects with ASCs seeded on bone morphogenetic protein 2 and scaffolds of β-TCP. Results from both studies show evidence that ASCs provide a non-invasive method of ossifying bone defects and allows for bony reconstruction without the adverse effects of traditional bone grafts, like donor site morbidity [2,9]. Other clinical trials include the use of ASCs to aid in bone defect treatments like upper arm fractures, alveolar cleft osteoplasty, maxillary sinus floor elevation, avascular necrosis of the hip, osteoarthritis of the ankle/knee/hip and spinal disc herniation [28]. Methods of ASC delivery included seeding autologous SVF from abdominal tumescent liposuction on porous silicate-hydroxyapatite microgranules with fibrin hydrogel implants; using a lateral ramus cortical bone plate with autologous ASCs from buccal fat pad mounted on nature bovine bone mineral; autologous SVF from abdominal tumescent lipo-aspiration seeded on a β-tricalcium phosphate implant; and intraarticular injection of platelet-rich plasma with autologous SVF from abdominal tumescent liposuction [28]. Although the trials involving the alveolar cleft osteoplasty did not show significant regeneration differences between the control and experimental groups, the trials using ASCs to treat upper arm fractures, maxillary sinus floor elevation, avascular necrosis of the hip, osteoarthritis of the ankle/knee/hip and spinal disc herniation did show significant results. Patients in the experimental groups showed a significant amount of bone healing and from these trials the safety of ASCs could be established since patients were monitored over long periods and showed no serious adverse effects or complications [28]. Further studies are needed to determine the most effective delivery method of ASCs to promote bony reconstruction, but past and current clinical trials show a promising future for the use of ASCs in cell-based bone regeneration therapies.

**Wound, Scar and Burn Healing**

Many clinical trials have shown conclusive evidence that ASCs are an effective aid in wound healing. ASCs are readily able to secrete cytokines and a variety of growth factors that have been shown to increase macrophage recruitment, improve vascularization and enhance tissue granulation at the site of the wound or burn [9]. The abundance of secretome groups allows for cell proliferation, differentiation, migration and fosters a healing microenvironment. Some of the known contributing growth factors include HGF, IGF-1, VEGF, Granulocyte Colony-Stimulating Factor (G-CSF) and Interleukin 8 (IL-8) [2,3,9]. Clinical trials within breast cancer patients have shown that the repeated transplant of purified autologous lipoaspirates improved the ultrastrucutal tissue characteristics with neovessel formation within radiation-induced lesions [2,3,9,29]. ASCs have also been used clinically within thromboangiitis obliterans and diabetic ulcer wounds, or wounds complicated by ischemia, to encourage collateral vessel formation [2-5,30]. Their angiogenic properties give them the ability to stimulate proliferation and the migration of endothelial cells that can lead to tube formation. Trials within animal models have utilized the aid of ASCs within aberrant scar formation and concluded that the injection of ASCs at the site of the scar reduces the surface area and improves color and pliability when compared to a control group [24]. In human clinical studies, repeated injections of large amounts of ASCs into firm scar tissue have resulted in scar softening and partial

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**DOI:** http://dx.doi.org/10.46889/JRMBR.2022.3101
healing [30,31]. ASCs also provide a solution to skin necrosis due to complications related to Hyaluronic Acid (HA) fillers. Tissue ischemia due to HA filler can be locally relieved using injections of harvested ASCs and results have been successful up to 7 days after the onset of symptoms, although ideally the treatment would begin before day 4-5 for the best results [30]. It is thought that the immunosuppressive and anti-inflammatory abilities of ASCs are due to the targeting of the inflammatory process associated with wound and scar formation, but further studies in humans need to be conducted for conclusive evidence [25].

**Peripheral Nerve Regeneration**

Patients can experience Peripheral Nerve Injury (PNI) as a result from complications with surgical procedures or traumatic injuries, but unlike neurons in the central nervous system, peripheral nerves can regenerate [32]. PNIs are both debilitating to the patient and costly for the healthcare system and until recently the treatment relied heavily on autografts [19]. This treatment option is effective but limited by donor availability, loss of nerve function and the potential of neuroma formation [33]. Using media-induced differentiation, isolated ASCs can be differentiated into Schwann Cells (SCs) and show no significant immunocytochemical property differences when compared to harvested SCs [19,32,33]. Although undifferentiated ASCs can be used to promote nerve regeneration, studies have shown that ASCs that were differentiated prior to clinical use showed higher neurotrophic function and therefore significant regeneration capabilities compared to those that were not differentiated [19]. ASCs have demonstrated the ability to produce numerous important growth factors including gliad-derived, nerve, brain-derived, glial cell-derived, insulin-like, hepatocyte and vascular endothelial growth factors [10]. This ability has benefits by giving ASCs the regeneration capabilities observed both *in-vitro* and *in-vivo* but further clinical trials are needed to determine the full potential of ASCs in nerve regeneration.

**Cosmetic Procedures**

For cosmetic purposes, CAL is favored over conventional lipoinjection due to the higher rate of success with transplantation. The transplantation of autologous fat using CAL has shown promising results for both soft tissue augmentation and skin rejuvenation. These procedures leave the patient with no incisional scar or complications associated with implants or other foreign material [18,24,27]. Facial changes linked to aging, like depressed features and loss of facial fullness, can be treated using lipoinjection [22,30,34-36]. Lipoinjection is also a popular solution to repair facial lipoatrophy that can be associated with several inherited or acquired diseases; these include patients with lupus erythematosus profundus and scleroderma en coup de sabre (en coup de sabre morphea), patients who suffer from Human Immunodeficiency Virus (HIV) infection or Parry-Romberg syndrome (hemifacial progressive atrophy or idiopathic hemifacial atrophy) and other diseases that also result in bony defects [22]. CAL is used to inject lipoaspirate, high in ASCs, to promote natural growth. Results in a facial
lipoinjection study showed that patients who received CAL had good or excellent clinical results compared to those who received non-CAL injections, experiencing only fair results or complications of necrosis in the injected tissue [22,25,36]. Clinical trials have also shown success in using CAL for cosmetic breast augmentation, with patients reporting improved contour, minimal complications and increased breast volumes [3,10,25,27,30,34]. CAL has been successfully utilized in both cosmetic breast augmentations and immediate augmentation after removal of breast implants [37]. One study found that the number of ASCs added to the lipoaspirate directly affected the outcome of breast augmentation. In those who received more ASCs results showed higher fat engraftment and a larger increase in breast volume, although there is no clinical consensus on the optimal number of cells per dose [27,37]. Studies have also shown that the use of autologous fat grafting has not been shown to increase the risk of breast cancer in patients receiving reconstructive surgery [14]. Similarly, ASCs provide a more natural alternative to synthetic fillers for use in buttock biomolding, in which results from clinical trials have been promising [38]. In less invasive aesthetic procedures, ASCs have been used to treat androgenetic alopecia through a hair restoration procedure involving ASCs from a Conditioned Medium (ASC-CM) [39]. Patients in clinical trials saw a significant increase in hair density when compared to baseline or placebo groups, which supports the use of ASC-CMs as an effective method of hair restoration [39]. The versatility of ASCs allows them to aid in numerous procedures throughout the field of plastic surgery and their capabilities have yielded promising results that put them at the forefront of many clinical trials.

**Conclusion**

ASCs have proven to be a promising tool for the future of regenerative medicine and cosmetic procedures. Current clinical trials have shown the effectiveness of ASCs to aid in the healing process of wounds and burns, regeneration of bone and proliferation of tissue allowing for successful long-term results in cosmetic procedures. Further research needs to be conducted comparing enzymatic and mechanical isolation of SVF and the affects, if any, it has on the long-term recovery, as well as clinical results a patient experiences. Although further clinical trials are needed to solidify the effectiveness of CAL compared to traditional lipoinjection, current and past trials have resulted in promising outcomes that favor CAL. Further research and clinical applications must be implemented to determine the full potential and limitations of ASCs, especially in relation to the ability of stem cells to promote tumor growth and metastasis.

**Conflict of Interest**

The authors declare that they have no conflict of interest.
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