Adipose-Derived Stromal Stem Cells

5

Lora L. Brown

Introduction

Autologous adipose-derived stem cells are an important source of therapeutic cells for patients suffering from traumatic, degenerative, or inflammatory disease processes. Clinical data have identified adipose tissue as an alternative source of mesenchymal stem cells (MSCs). Stromal vascular tissue derived from adipose tissue contains a subset of tissue that is different from that found in blood cells. Adipose stromal tissue contains a subset of multipotent progenitor cells with adipogenic, chondrogenic, osteogenic, and myogenic differentiation potential [1].

Adipose tissue is abundant, easily accessible, and easily obtainable via lipoaspiration with little patient discomfort. Adipocytes make up the bulk of adipose tissue. A heterogeneous cell population called the stromal vascular fraction (SVF) surrounds the mature adipocytes. The SVF includes adipose stromal stem/progenitor cells (ASCs), pericytes, mature and immature vascular endothelial cells, fibroblasts, and hematopoietic-lineage cells [2] (Table 5.1).

A large body of in vitro research shows that adipose-derived stem cells are located within the perivascular niche within the stromal vascular fraction. The stromal vascular fraction (SVF) parallels the mononuclear cell fraction obtained from bone marrow–derived stem cells [3] (Table 5.2). Both tissue sources possess regenerative cellular potential, but 1 mL of adipose tissue contains 300 to 500 times more MSCs than 1 mL of bone marrow aspirate [4]. The cell populations present in the SVF include hematopoietic-lineage cells (stem and progenitor cells, granulocytes, monocytes, lymphocytes), endothelial cells, pericytes, and stromal cells. Collectively, these cell populations possess many advantageous characteristics, including immunomodulatory, anti-inflammatory, antiapoptotic, angiogenic, and mitogenic properties. They also resist scar cascade initiation. These cells accomplish regenerative functions via complex secretion and signaling of growth factors and cytokines. These paracrine effects, as well as direct cell-to-cell interactions, exert great effects on local tissue repair by activating endogenous progenitor cells previously dormant in the affected tissue [1, 5–8]. Consequently, there is a decrease in inflammation and pain, as well as regeneration of tissue in the damaged areas.

It should be noted that stem cell paracrine potential is thought to vary based upon cell tissue origin. Cell surface markers of mesenchymal stem cells have demonstrated

Table 5.1 Commonly used markers to characterize cell populations in SVF

Cell type	Phenotype	Proportion of nonheme (CD45−) nucleated cells
Stromal/ preadipocytes	CD31−, CD34+, CD146−/+, CD90+	67.6 ± 29.7%
Endothelial progenitor	CD31+, CD34+, CD146+, CD90+	5.2 ± 6.1%
Endothelial mature	CD31+, CD34−, CD146−, CD90−	Variable with harvest technique
Pericytes	CD31−, CD34−, CD146+, CD90+	0.8 ± 0.7%

Table 5.2 Comparison of bone marrow–derived and adipose-derived stem cells

Bone marrow aspirate concentration (BMAC)	Adipose-derived stem cells (SVF)
Easy to obtain	Moderate difficulty to obtain
Bone marrow aspiration	Tumescent liposuction
Centrifuge and remainder of materials come in commercially available kits	Flow hood, incubator, tissue culture hood, plus equipment that is typically purchased a la carte
Takes less than an hour to harvest cells, process, and inject to target region	Can take an hour just to harvest cells
Lower nucleated cell concentrations	Higher nucleated cell concentrations
Progenitor and stem cell concentrations unpredictable and typically lower	Progenitor and stem cell concentrations predictable and much higher

L. L. Brown (✉)
TruWell, PLLC, St Petersburg, FL, USA

C. W Hunter et al. (eds.), *Regenerative Medicine*, https://doi.org/10.1007/978-3-030-75517-1_5

region-specific variation. Understanding the metabolic activity mechanisms within different stem cell tissue (SVF) niches is a current area of research interest that may provide a clearer understanding of the cellular maintenance of mesenchymal stem cells as well as their regenerative and pro-angiogenic potential.

Although limited, human studies involving MSCs for the treatment of osteoarthritis are promising. Mesenchymal stem cells derived from bone marrow aspirate and percutaneously injected into subjects with MRI-proven degenerative joint disease of the knee showed statistically significant cartilage and meniscus growth on MRI, as well as increased range of motion and decreased modified Visual Analog Scale (VAS) pain scores at 21 weeks after the injection [9]. Emadedin et al. treated six female subjects with osteoarthritis of the knee who were candidates for knee replacements with bone marrow–derived MSCs and found improvements in pain, functional status, and walking distance 6 months post-injection [10]. MRI images at baseline and 6 months postinjection demonstrated an increase in cartilage thickness, extension of repair tissue over the subchondral bone, and a considerable decrease in the size of edematous subchondral patches. In a similar study, autologous MSCs derived from adipose tissue were administered to 18 patients with osteoarthritis of the knee. The results showed that intra-articular injection of 1.0×10^8 adipose-derived MSCs into the osteoarthritic knee improved function and pain of the knee joint without causing adverse events, and it reduced cartilage defects by regeneration of hyaline-like articular cartilage [11].

Another area of interest in regenerative medicine is the treatment of degenerative disc disease. Researchers have demonstrated that intervertebral discs contain an endogenous stem cell population of skeletal progenitor cells displaying osteogenic, adipogenic, and chondrogenic characteristics, which are the same characteristics shared by MSCs derived from both bone marrow and adipose tissue. Mesenchymal stem cell implantation has been shown to stimulate nucleus pulposus cell proliferation and MSC chondrogenic differentiation, as well as increasing production of cytokines, particularly transforming growth factor-beta [12, 13].

Animal studies for the treatment of disc degeneration have demonstrated that MSCs injected into the nucleus pulposus not only survive but proliferate in canine, porcine, and rabbit models. The results of these studies also showed that the transplanted stem cells influenced the production of extracellular matrix proteins, including aggrecan, proteoglycans, and type I and type II collagen. Most importantly, these injections resulted in the preservation of both water content and height in the damaged disc [14–17].

Human studies utilizing stem cells for the treatment of degenerative disc disease are promising. Orozco et al. conducted a pilot study utilizing autologous culture-expanded bone marrow mesenchymal cells for intervertebral disc repair [18]. Ten subjects were followed for 1 year to evaluate back pain, disability, and quality of life. Magnetic resonance (MR) imaging measurements of disc height and fluid content were also performed. Results confirmed feasibility and safety. Patients exhibited rapid improvement of pain and disability at 85% of maximum in 3 months. MRI scans showed that although disc height was not recovered, water content was significantly elevated at 12 months.

Pettine et al. investigated the use of autologous bone marrow concentrate for the treatment of discogenic pain [19]. Twenty-six subjects received percutaneous injections in one or two intervertebral discs and were evaluated using MR imaging, the Oswestry Disability Index (ODI), and VAS. Results showed a substantial reduction in pain of 69.5% on the ODI and 70.6% on the VAS. Eight of 20 patients improved by one modified Pfirrmann grade at 1 year. Furthermore, recent basic research and preclinical studies have revealed that the use of adipose-derived MSCs in regenerative medicine is not limited to mesodermal tissue but extends to ectodermal and endodermal tissues and organs as well [20].

Although there are little data to support the wide array of disease processes treated with stem cell therapy, the evidence is growing exponentially. Physicians around the world utilize adipose-derived MSCs to treat some of the most troubling maladies. Today these therapies are limited to "last resort treatments" for those who can afford them, but some day, regenerative therapies will likely be at the forefront of advanced medical therapies.

Indications

In the field of musculoskeletal medicine, adipose stem cell therapy has been used in the treatment of muscle, tendon, and ligament injuries as well as joint arthritis. Painful degenerative disc disease, facet arthritis, and sacroiliac joint pain are also reasonable applications for this therapy.

Although there are no clear treatment protocols defined for the use of adipose stem cell therapy, the current standard of care preserves this treatment for those patients who have failed conventional treatment options or who are not candidates for conventional treatment options.

Musculoskeletal Conditions Treated with Adipose-Derived MSCS

- Joint osteoarthritis and rheumatoid arthritis
- Tendon, ligament, or meniscal incomplete tears
- Shoulder or hip labral tears

- Rotator cuff disease
- Degenerative disc disease
- Facet and sacroiliac joint disease

An evolving body of evidence suggests adipose-derived stem cells are also therapeutic for systemic autoimmune and inflammatory diseases. Although these diseases may fall outside the scope of this book, it important to understand the breadth of potential therapeutic applications of this treatment.

Chronic Conditions Treated with Adipose-Derived MSCS

- Osteoarthritis
- Rheumatoid arthritis
- COPD
- Heart failure
- Multiple sclerosis
- Alzheimer's disease
- Parkinson's disease.
- ALS
- Ulcerative colitis
- Poorly healing wounds
- Spinal cord injury
- Post-stroke
- Diabetic neuropathy
- Erectile dysfunction

Microanatomy and Biochemistry

The mesenchymal stem cells (MSCs) in adult adipose tissue are powerful progenitor cells that have the amazing capacity to differentiate into specific cell types that generate mesenchymal tissue including bone, cartilage, tendon and ligament, muscle, fat, dermis, and other connective tissues. These cell types include osteoblasts, chondrocytes, myoblasts, and fibroblasts, the very lineages that evolve to many of the musculoskeletal tissues targeted in regenerative medicine [1, 6–8, 20].

The characterization of adipose-derived MSCs has been described in the literature [21, 22]. The stromal vascular fraction (SVF) is composed of the following:

- Hematopoietic stem cells, 2%
- Pre/endothelial cells, 7%
- Pericytes/smooth muscle cells, 2%
- Fibroblasts, 47%
- Other (macrophages, various blood cells), 33%
- Adipose-derived stem cells, 2–5%

Adipose-derived MSCs have trophic, immunomodulatory, and antimicrobial functions. Included in the trophic functions are angiogenic, mitogenic, antiapoptotic, and antiscarring properties [1, 7, 8, 20–22].

Some of the cytokines found in the adipose-derived SVF include high levels of expression of several growth factors:

- Hepatocyte growth factor (HGF): Plays a major role in embryonic organ development; in adult, organ regeneration and wound healing.
- Vascular endothelial growth factor (VEGF): Stimulates growth of new blood vessels.
- Placental growth factor (PGF): Involves in angiogenesis and vasculogenesis.
- Transforming growth factor-beta (TGFβ): Controls proliferation, cellular differentiation, and other functions.

Also found are moderate levels of expression of other factors:

- Fibroblast growth factor (FGF-2): Involves in wound healing and angiogenesis.
- Angiopoietin (Ang-1 and Ang-2): Promotes angiogenesis and formation of blood vessels.

Mesenchymal stem cells demonstrate the ability to release bioactive molecules that are immunoregulatory. They respond to environmental signals that are tissue specific. In response to these signals, MSCs secrete a wide array of paracrine factors that create a regenerative milieu that possesses trophic regenerative properties. Consequently, it is felt that the beneficial impact of adipose-derived MSCs on various tissues and organs may be due to soluble factors produced by the cells, rather than to their tissue differentiation capabilities. Moreover, it has also been shown that the soluble factors secreted by adipose-derived MSCs can be modulated by exposure to different agents, giving promise to the field of tissue engineering [1, 7, 8, 20, 22, 23].

Adipose-derived MSCs have an inherent ability to locate damaged tissue. Their response to molecular signaling within the body has been demonstrated in studies using radionucleotide-tagged cells [22].

Regulatory Status

Adipose tissue is a human cell, tissue, and cellular and tissue-based product (HCT/P), which is defined in Title 21 of the Code of Federal Regulations Part 1271 (21CFR 1271.3(d)) as articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient. Because of its unique nature, HCT/Ps have been regulated by the FDA through a tiered,

risk-based approach. The FDA is authorized to apply the requirements in the Federal Food, Drug, and Cosmetics Act and the Public Health Service Act (PSA) to those products that meet the definition of drug, biologic, or device. Some HCT/Ps that meet specific criteria do not require premarket review and approval. In order to meet those criteria, specific registration, manufacturing, and reporting steps must be followed in order to prevent the introduction, transmission, and spread of communicable disease. The steps to qualify as an "exempt" HCT/P product are found in PSA Section 361.

The FDA released two draft guidance documents in 2014 that were finalized in 2017 that addressed specific definitions used to define a Section 361 "exempt" HCT/P product [24, 25]. The draft guidance documents further defined "same-day surgical procedure," "minimal manipulation," and "homologous use" with a specific mandate that a HCT/P that qualified as a Section 361 product must meet all three of these criteria.

To this day, there exists much contention between regenerative stem cell clinicians and the FDA regarding the definition of minimal manipulation, homologous use, and adipose tissue. The FDA contends that the use of adipose tissue via enzymatic digestion, ultrasonic cavitation, or other processing methods was considered more than minimal manipulation. Therefore, the process of SVF production was not considered to satisfy the three criteria of 21 CFR 1271 Section 361 HCT/P: homologous use, minimal manipulation, same-day surgical procedure. Many clinicians disagree with this interpretation as adipose tissue is defined not only as a structural support tissue but also as a metabolic endocrine tissue with endocrine and paracrine functions.

Continued widespread use of adipose as a HCT/P product that meets Section 361 exemption prompted the FDA to publish two additional guidance documents in 2017 [26, 27]. In line with the Twenty-First Century Cures Act passed in December 2016, the FDA published these documents as part of comprehensive FDA policy framework to address plans to support and expedite the development of regenerative products, including HCT/Ps. The Twenty-First Century Cures Act was designed to help accelerate medical product development and bring new innovations and advances to patients who need them faster and more efficiently and to simplify and streamline its application of the regulatory requirements for devices used in the recovery, isolation, and delivery of *regenerative medicine advanced therapies* (RMATs), including combination products [28, 29].

The FDA proclaimed its intent to apply a risk-based approach to enforcement of cell-based regenerative products through November 2020, taking into account how products are being administered as well as the diseases and conditions for which they intend to be used. That discretion will not be afforded to those that pose significant potential patient safety concerns.

There are currently several companies that have applied for consideration of their products as Investigational New Drug (IND). And several others are pursuing New Drug Applications (NDA) and Biologics License Applications (BLA).

Adipose-derived stem cells thus are considered and regulated as a drug, device, and/or biologic product. The FDA has clearly stated its position against adipose stem cell therapy meeting the necessary criteria for exemption of premarket review and approval and thus does not qualify for 361 exemption status. Even though clinicians continue to offer adipose stem cell therapy to their patients, there are potential repercussions. With new accelerated pathways for regenerative medicine companies to pursue FDA approval, it is almost certain that new and improved adipose therapies will evolve.

Basic Concerns and Contraindications

The clinical application of cell-based therapies is somewhat controversial. Considered experimental, the therapy is not FDA-approved as of 2019. These facts must be disclosed to all prospective patients. Potential patients should also be informed that their treatment might prevent them from participating in future clinical research studies.

Cell-based therapies are minimally invasive, relatively safe approaches to complex diseases, though the lack of conclusive evidence creates some questions as to their safety and efficacy. It is estimated, however, that hundreds of thousands of autologous stem cell treatments are done per year worldwide, with a paucity of reported complications.

There is some variation in the number of stem cells present in various donor sites and with donor age [30–32]. In general, the most efficient methods can isolate about 500,000–1,000,000 cells per gram of lipoaspirate tissue with a >80% viability. The number of viable cells required for treatment of a particular condition is unknown because there are insufficient data to establish a reliable dose versus effect relationship.

Angiogenesis and mitosis are effective outcomes of cell-based therapies, so there is a theoretical risk of tumorigenesis or increased growth of preexisting cancers. This result has not been seen clinically. Hernigou et al. reported no increased cancer risk in 1873 patients who were observed for an average of 12.5 years after treatment with autologous cell-based therapy using bone marrow–derived stromal progenitor cells [33]. Nevertheless, many physicians consider preexisting solid tumor disease a contraindication to stem cell therapy.

Contraindications for the use of autologous stem cell therapy in musculoskeletal medicine include retracted complete ligament or tendon tears and loose bodies in the articular space. In these cases, surgical therapy is warranted.

The use of stem cell therapy within the spine is nascent. Proper indications and contraindications will be developed as the therapy gains wider utilization, but it is clear that some findings within the spine would constitute a contraindication. These include spinal instability, disc extrusion, Modified Pfirrmann Grade VIII disc disease, critical spinal stenosis, and spinal infection.

Other conditions considered to be contraindications to autologous adipose stem cell therapy are preexisting local or systemic infection, severe cardiovascular disease, and blood dyscrasias.

Preoperative Considerations

Age, general health, nutritional status, and the availability of adipose tissue should be considered when evaluating a patient for autologous stem cell therapy. In patients with advanced age or nutritional or medical compromise, autologous therapy may not be the best option, and an allogeneic approach can be considered. Emaciated patients or high-performance athletes may not have an adequate volume of adipose tissue. In those cases, alternative treatments should be considered.

The health of each patient should be assessed preoperatively. Patients should be encouraged to stop smoking 4 to 6 weeks before treatment. Heavy alcohol consumption should be avoided. Nutrition should be optimized with clean, whole foods and nutritional supplementation.

NSAIDs should be avoided at least 2 weeks before and 4 weeks after autologous or allogeneic stem cell therapy. Steroid injections should be avoided for 4 weeks before and after treatment. Within the orthopedic literature, NSAIDs have been linked to impaired fracture healing and abnormal chondrocyte differentiation. Nonsteroidal anti-inflammatory medications and steroids have also been demonstrated altered stem cell (MSC) gene expression, decreased cell proliferation, and altered cell differentiation [34–36].

The patient's medical condition will determine the amount of adipose to be aspirated. Most systems utilize approximately 60 cc of adipose tissue to recover a therapeutic dose of SVF, which should contain 50 to 100 million stem cells. An adequate adipose harvest site must be selected. The abdomen is commonly used, but in some instances, one must resort to the flank or "love handles," the hips, or thighs. Careful examination of the area should include notation of any prior operative procedures that may have produced scar tissue within or near the lipoaspiration area. Topographical, superficial skin markings performed preoperatively with the patient standing may provide a useful guide during the procedure. Although this is not a cosmetic procedure, one should attempt to provide a symmetric and appealing outcome.

A proper procedure consent should be completed and signed by the patient on the day of the procedure, prior to any sedative medication, including the following points:

- Consent for tumescent anesthesia
- Consent for lipoaspiration
- Consent for reintroduction of the final product, whether that be a joint injection; a muscle, tendon, or ligament injection; or an intravascular or intrathecal injection
- Disclosure that the procedure is experimental
- Disclosure that the procedure is not FDA-approved
- Acknowledgment that a successful outcome is not guaranteed
- Disclosure that the treatment may eliminate the patient's candidacy for future clinical research studies

Assess whether the patient would like to "bank" or cryopreserve some cells. Several FDA-listed tissue banks will cryopreserve a patient's adipose-derived MSCs for a fee. Theoretically, the tissue that is stored will always be more youthful and beneficial than tissue available in the future. Most tissue banks require 60–100 mL of adipose to be shipped overnight. The adipose tissue is processed, and the cells are expanded and cryopreserved until future need. Currently, expanded cell products are considered highly processed and consequently are subject to the Public Health Safety Act, Section 351. For such tissue to be used in the United States, it would need to be licensed by the FDA as a biological drug [37].

Preoperative intravenous antibiotics may be considered, as well as an anxiolytic.

Equipment

Figure 5.1 shows some of the equipment needed for lipoaspiration:

- 14 g/25 cm garden spray infiltration cannula
- 3 mm/25 cm Mercedes cannula
- 60-cc syringe snap lock
- Syringe caddy
- 2-quart stainless steel bowl
- 60-cc Luer lock syringes × 4
- 60-cc Toomey syringe × 2
- #11 blade scalpel
- 10-cc syringe
- 18-gauge 1-inch needle
- 25-gauge 1.5-inch needle
- Sterile back cover drape
- Sterile half drape
- Sterile prep kit (povidone-iodine or Hibiclens®, Mölnlycke Health Care, Norcross, GA)

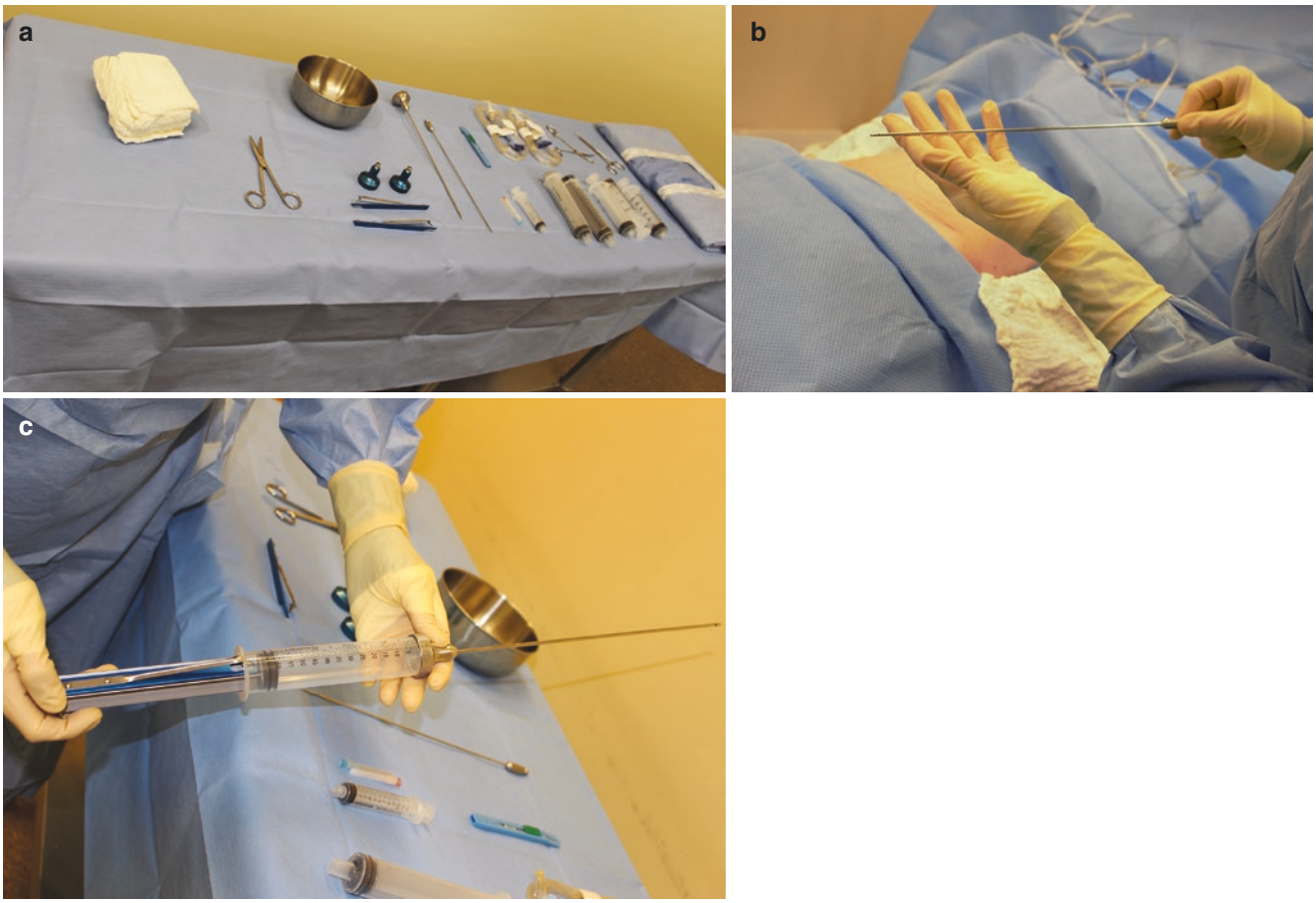

Fig. 5.1 (**a**) Back table setup for lipoaspiration procedure; (**b**) irrigation cannula; (**c**) lipoaspiration cannula with snap lock

- Sterile surgical marking pen

Also to be used are several medications and some items of laboratory and tissue culture equipment:

- 0.9% sodium chloride IV solution (1000 mL)
- 8.4% sodium bicarbonate, 1 mEq/mL (50 mL vial)
- Lidocaine HCl 2% (50 mL vial)
- Epinephrine 1:1000 (30 mL vial)
- HEPA-filtered Class 100 laminar flow biological cabinet
- Centrifuge
- Dry block incubator or incubator shaker
- Disposable manual stem cell isolation kit

Technique of Lipoaspiration

Rodbell and James pioneered the initial techniques used to isolate cells from adipose tissue in the 1960s. The procedure has evolved to become a safe and minimally invasive procedure [38–40]. Today the isolation procedure includes the following steps:

- Tumescent liposuction, which finely minces tissue fragments (dependent on the size of the cannula)
- Washing to remove hematopoietic cells
- Enzyme or mechanical digestion
- Centrifugation to separate the SVF
- Isolating SVF with washing cells, centrifugation, and cell strainer
- Cells (SVF) prepared in the final solution

Preparation of Tumescent Anesthetic Fluid

The tumescent technique uses the standard anesthetic solution used for liposuction procedures. Tumescent fluid premixed on the day of the procedure is infiltrated into the subcutaneous tissue in order to anesthetize the procedure site locally. The amount of tumescent fluid used is calculated based upon the amount of adipose being harvested; it is limited by the maximum lidocaine dose based upon the patient's weight (4.5 mg/kg; 7 mg/kg when combined with epinephrine). The safe dosage for tumescent lidocaine was shown to be 35 mg/kg by Klein in 1990, and this has become standard of care for liposuction procedures [41].

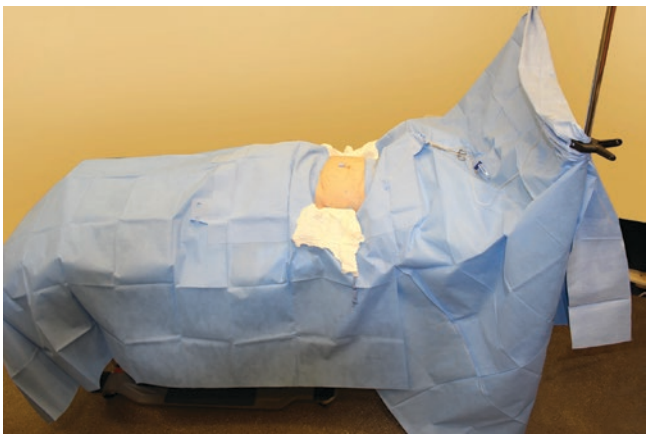

Fig. 5.2 Patient positioning and draping

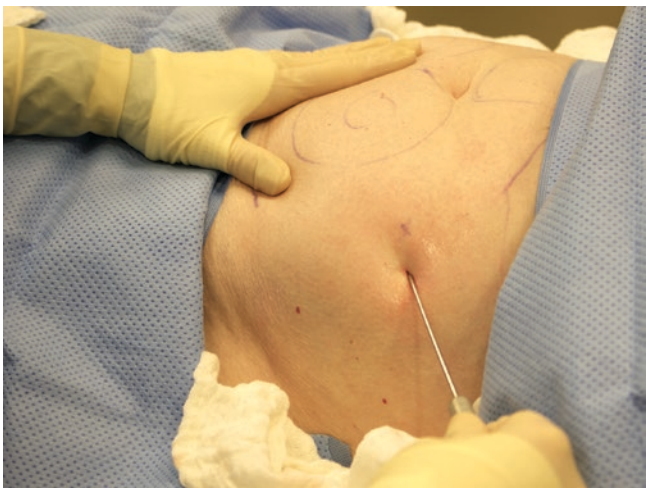

Fig. 5.3 Infiltration of tumescent fluid

- For harvesting small amounts of adipose tissue (i.e., 60–120 mL), a 0.1% tumescent solution may be utilized. Into a 1000-mL bag of 0.9% sodium chloride, introduce the following using the sterile technique:
  - 50 mL lidocaine 2%
  - 1 mL epinephrine 1:1000
  - 10 mL sodium bicarbonate 8.4%
- For harvesting large amounts of adipose tissue (>120 mL), a 0.05% tumescent solution can be utilized. Into a 1000-mL bag of 0.9% sodium chloride, introduce the following using the sterile technique:
  - 25 mL lidocaine 2%
  - 1 mL epinephrine 1:1000
  - 8 mL sodium bicarbonate 8.4%

The tumescent solution should be mixed on the same day as the procedure, and the epinephrine should be added immediately prior to use. The bag should be clearly identified and dated.

Infiltration of Tumescent Anesthetic Fluid

The patient is taken to the procedure suite and positioned supine for abdominal adipose harvesting or posterior or lateral decubitus for flank/hip adipose harvesting. Appropriate monitoring is placed. Sterile prep and drape is performed over the lipoaspiration site (Fig. 5.2). The port placement should be considered. If the abdomen is the harvest site, the port sites should be asymmetrically placed bilaterally at the anterior axillary line, at the level of the anterior iliac spine. Place a local anesthetic skin wheal at these sites. Using a #11 blade scalpel, make a 5-mm skin incision. The tumescent fluid is then infiltrated subcutaneously using the 14 gauge garden spray infiltrating cannula throughout the area of lipoaspiration. The tumescent fluid IV bag may be hung on an IV pole with pressure bag–assisted gravitational flow. As an alternative, the tumescent fluid may be delivered manually with a 60-cc syringe.

Tumescent fluid infiltration should be delivered slowly and evenly throughout the tissue. The irrigational cannula must remain parallel with the abdominal wall to avoid any unintentional transabdominal or peritoneal injury. Adequate infiltration is appreciated when the skin appears firm with turgor. There may be blanching, demarcating vasoconstriction associated with the epinephrine (Fig. 5.3).

Collection of Lipoaspirate

Once the tumescent fluid has been infiltrated, lipoaspiration is conducted with a 3 mm/25 cm Mercedes cannula attached to a 60-cc Toomey syringe, with a moderate amount of suction pressure. This is obtained by pulling back the syringe plunger after the cannula has been placed subcutaneously. Using a “snap lock” or “Johnnie snap” will support the plunger in this position while you work (Figs. 5.2 and 5.4).

The cannula is manipulated in a fanlike manner throughout the targeted tissue as the lipoaspirate is collected. The nondominant hand should be used as a guide to feel the tip of the cannula, ensuring that the cannula tip is not too superficial and does not extend beyond the intended treatment area (Fig. 5.4). Using this technique, deeper areas are aspirated first, followed by more shallow areas. Take care not to repeatedly course a specific area, as doing so may cause dimpling of the skin.

Continue to suction the aspirate until the syringe is full. Then place it upright in a syringe rack to allow the fat to rise above the supernatant fluid. Drain any supernatant fluid into a sterile stainless steel bowl and continue until the desired volume of fat has been harvested (Fig. 5.5).

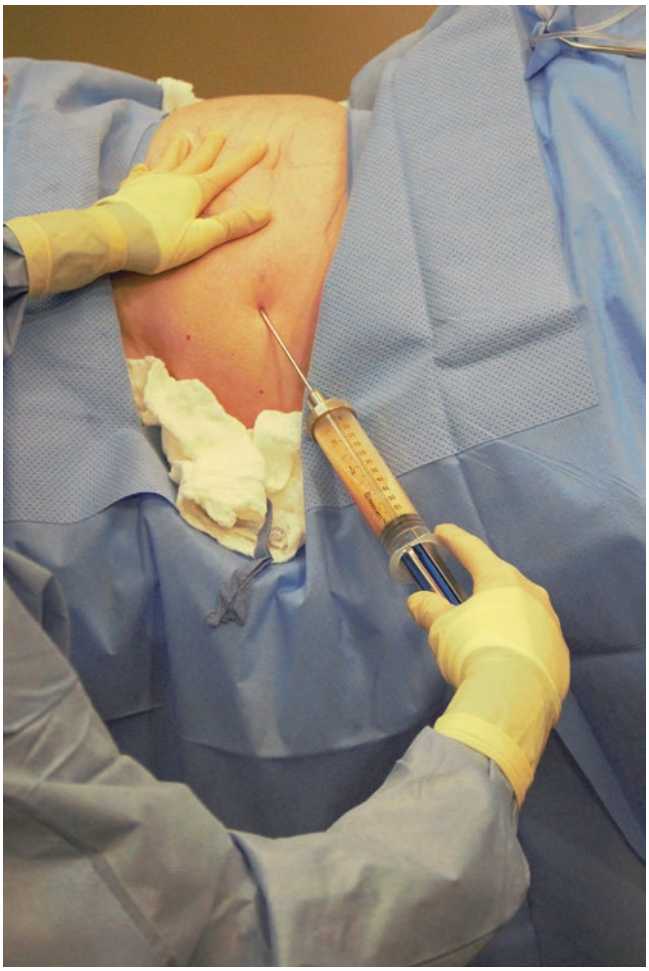

Fig. 5.4 Collection of lipoaspirate

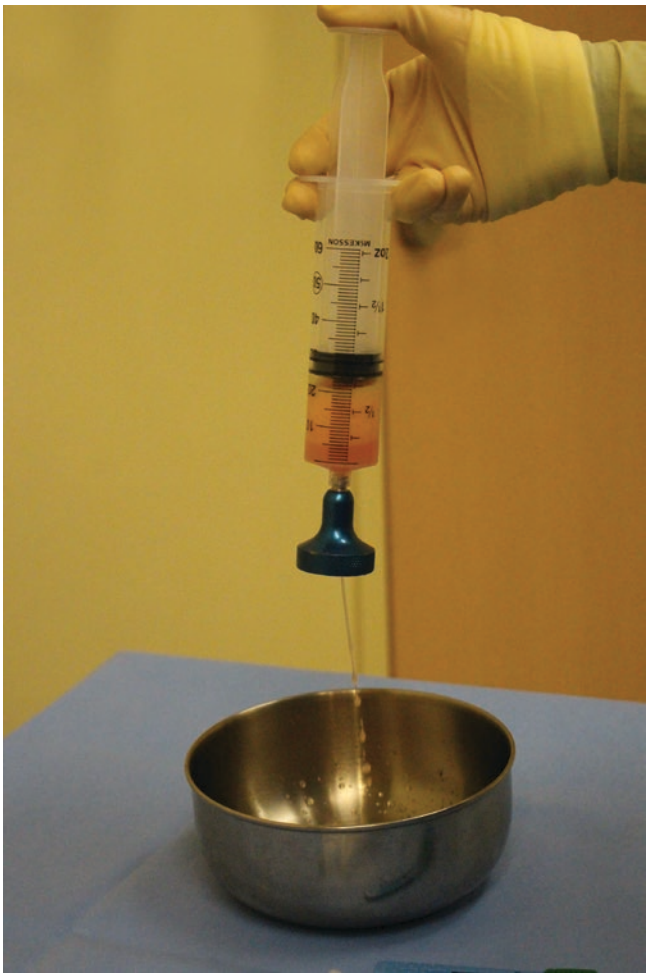

Fig. 5.5 Separation of fat from supernatant fluid

Once collected, the harvested fat should be transferred to the processing area in a closed system. If using a syringe, cap the syringe for transport (Fig. 5.6).

Post-Procedure Care

- Gently express any excess tumescent fluid through the port sites.
- Close the port sites with steri-strips.
- Apply absorbent dressings over the port sites.
- Apply a compression garment. This can be a compression bodysuit for patients with thigh or hip lipoaspiration sites, but a simple abdominal binder will suffice for most patients. The patient should be instructed to wear the compression device continuously for the first 72 hours, and then daily for the next 3 to 4 days. Compression aids in hemostasis, improving post-procedure bruising and discomfort, and helps with post-procedure aesthetics.
- Transfer the patient to recovery and monitor vital signs.

Adipose Processing: Enzymatic and Mechanical

There are two generally accepted means for isolation of the SVF from adipose tissue: mechanical and enzymatic. Both methods are equally safe, but there are differences to be noted when choosing between them. Mechanical isolation is less costly and quicker to perform, but the end product will contain a higher concentration of blood mononuclear cells and fewer progenitor cells [42]. When contemplating using smaller quantities of adipose tissue for SVF extraction, the mechanical method may be considered. Enzymatic isolation, on the other hand, has been shown in studies to demonstrate a significantly greater efficiency in the separation process through a consistent and predictable digestion of the extracellular matrix (Table 5.3). For this reason, the authors advocate the enzymatic isolation method, as outlined here:

- Harvested adipose tissue should be processed in a clean setting. All specimens should be clearly marked with

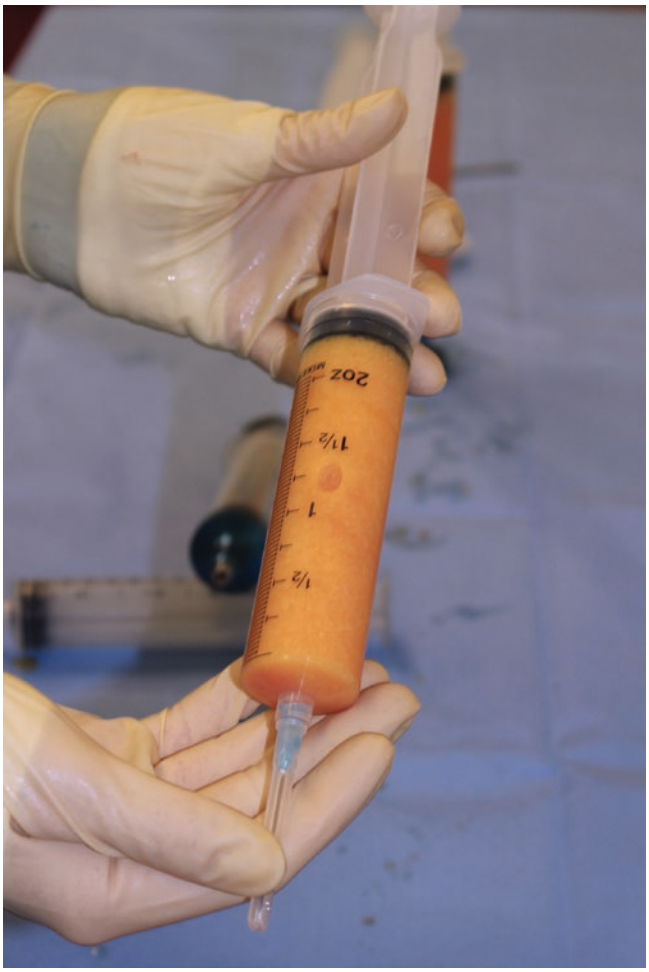

Fig. 5.6 Transport of the harvested adipose

Table 5.3 Comparison of mechanical vs. enzymatic isolation methods for extracting the SVF from harvested adipose tissue

	Mechanical isolation	Enzymatic isolation
Time to perform	15–30 minutes	2–3 hours
Cost	No added cost	$2–$5 per gram
Cell count (nucleated cells per cc of lipoaspirate)	1.0×10^4 to 2.4×10^5	1.0×10^5 to 1.3×10^6
Progenitor cell concentrations	Lower	Higher

patient identifiers. We recommend that all tissue handling outside of the sterile procedure suite occur under a Class 100 HEPA-filtered laminar flow biological cabinet using an aseptic technique (Fig. 5.7).

- Several companies offer proprietary formulas including protocol steps and unique digestive enzymes, which are packaged in disposable kits. The basic steps universally utilized to isolate adipose stem cells involve a cell wash and collagenase digestion, followed by centrifugal separation and filtration to isolate the single-cell SVF from the primary adipocytes.
- The SVF is then resuspended in a carrier solution for final treatment. The carrier solutions include autologous platelet-rich plasma and preservative-free normal saline. Autologous platelet-rich plasma is the author's preferred carrier solution for musculoskeletal, intrathecal, or intravascular therapeutic applications. The total resuspension volume may range from 2 to 10 cc depending on the site of treatment.

Reintroduction of the Adipose Stem Cell Product

For musculoskeletal applications, the patient is transferred back to the clean procedure suite and positioned appropriately for the injection, with appropriate monitoring. The injection (whether intra-articular or soft tissue) should be done with direct visualization utilizing fluoroscopy or ultrasound. A 22-gauge or larger bore needle should be utilized to prevent shear force–induced cell rupture. Note that contrast material is cytotoxic and should not be used. Additionally, many local anesthetics are cytotoxic. One percent lidocaine is well tolerated.

For systemic applications, the resuspended stem cell solution will be injected into a peripheral vein through an IV catheter or needle. The injection should be done as an IV push slowly over 5–10 minutes. The patient's pulse, oxygen saturation, and blood pressure should be monitored before, during, and after the injection.

For intradiscal, facet joint, or sacroiliac joint injections, the patient is taken to the clean procedure suite and positioned prone with monitors applied. Fluoroscopy or ultrasound guidance should be used to confirm the accurate needle placement.

For intrathecal applications, the patient is taken to the clean procedure suite and positioned in a prone position with monitors applied. Sterile prep is carried out, and the patient is draped for a lumbar or cervical fluoroscopically guided intrathecal injection. A 22-gauge spinal or Tuohy needle may be used. Use minimal contrast, as larger amounts of contrast could be cytotoxic to the MSCs. Confirmation of the intrathecal location is demonstrated by CSF flow through the hub of the needle.

Most commercially available adipose harvesting and processing systems include a filtration step. All final products should be filtered through a 100-micron filter to prevent potential embolic events.

Post-Procedure Care and Potential Complications

- Neither NSAIDS nor steroids are recommended for 4 weeks after treatment [34–36].
- The anti-inflammatory properties of the treatment may result in positive effects within the first couple of weeks in

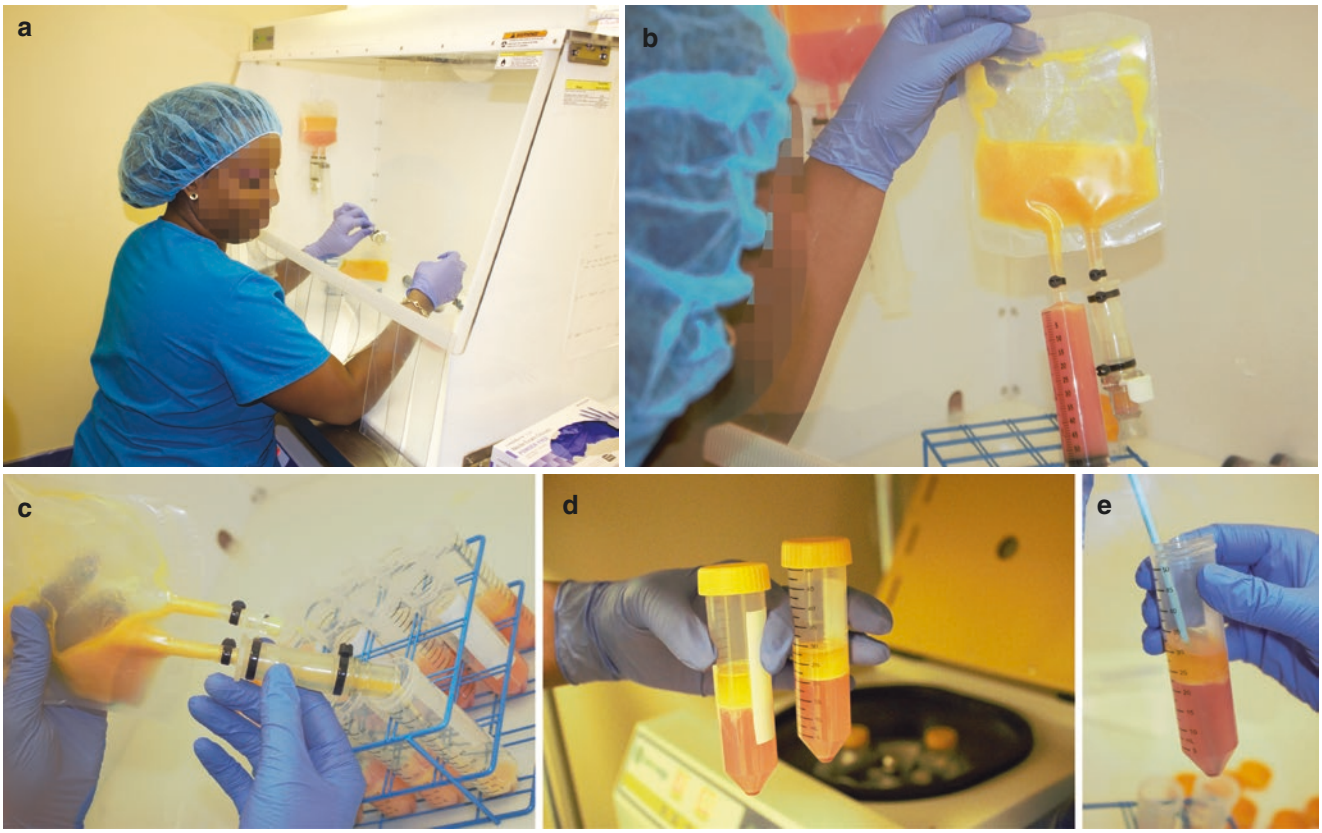

Fig. 5.7 (**a**) Processing of harvested adipose tissue under a laminar flow hood; (**b**) processing of harvested adipose tissue: washing the adipose; (**c**) processing of harvested adipose tissue: separating adipose into 50-ml conicals for centrifugation; (**d**) processing of harvested adipose tissue: after centrifugation the adipose has separated into the SVF at bottom and adipose at top; (**e**) processing of harvested adipose tissue: collecting the SVF pellet from the bottom of the conical

some cases, but the true therapeutic results may take 3 to 6 months to be realized.

- Normal light activity is recommended for the initial week after the procedure. A return to light exercise is recommended at 6 weeks after treatment.
- Many in the field believe that repeat treatments may be needed for many patients with severe local or systemic disease processes, though there is no research to support this idea. Autologous stem cell therapy may not offer a cure, but it certainly may offer a nonpharmacological treatment alternative.
- Although autologous adipose stem cell therapy is considered a safe same-day procedure, there are potential complications.
  - Infection due to poor sterile technique or contamination of the tissue product is possible. Fortunately, MSCs have demonstrated an antibiotic propensity to protect against this possibility.
  - Harvest site pain, soreness, or bruising may occur but is usually mild and can be treated with supportive therapy such as ice, acetaminophen, or analgesics. If symptoms persist, have the patient come in for a clinical evaluation.
  - Injection site pain, soreness, or bruising is also usually mild and responsive to supportive care. If persistent, have the patient come in for a clinical evaluation.
  - Skin dimpling or other cosmetic disfigurement is possible. It is always important to practice good techniques during lipoaspiration. Avoid excessive aspiration in any given area.
  - Because you are using the patient's own tissue in this therapy, there is no risk of rejection, but if you are processing tissue samples from multiple patients on the same day, there is a risk of injecting the wrong sample into a patient. Always clearly label all specimens through the entire isolation process.

Clinical Pearls

- Standard universal precautions should be followed by all personnel with potential exposure to any patient tissue.
- There have been case reports of transient hypertension and tachycardia and/or symptoms of lightheadedness, flushing, or headache upon systemic intravascular

injections. Always monitor your patient and have oxygen and supportive medications available.

- Although it is highly unlikely that you will ever need it, have a crash cart and airway resuscitative equipment available. Many of your patients may have multiple comorbidities.
- Contrast, antibiotics, and many local anesthetics have been shown to be cytotoxic to mesenchymal stem cells. Use only 1–2% lidocaine, which has been shown not to be cytotoxic, and limit the amount of contrast injected when possible.
- Inject the final product with needles and catheters of 22-gauge or larger bore. This bore size does not disrupt the cell structure.
- Proprietary cell isolation techniques can provide safe, legal methods to consistently harvest approximately 50–100 million cells per 60–100 mL of adipose tissue, with reproducibility and validated analysis.
- Cell yield can be affected by several factors:
  - Surgical technique.
  - Location of fat.
  - Enzymatic digestion: Enzymatic digestion times and concentrations strongly modify the yield and viability of cells.
- Consider in advance the volume of injectate you will need for each area treated when performing your final resuspension. A small joint such as a finger or facet joint will only accommodate 1 to 2 mL of fluid, whereas a large joint such as a knee may require 6 to 8 mL or more.
- I frequently recommend an intravascular dose as well as an intra-articular dose in patients with osteoarthritis. Mesenchymal stem cells have demonstrated a unique homing ability. When introduced intravascularly, they make their way to the damaged tissue via cell signaling mechanisms.
- Although not endorsed by the FDA, there is a theoretical benefit for the use of intravascular stem cell treatments for autoimmune and inflammatory diseases, as well as for prevention and longevity.
- Do not advertise or make therapeutic claims with regard to this therapy. The FDA is hypervigilant regarding such public statements.

References

1. Murphy M, Moncivias K, Caplan AI. Mesenchymal stem cells: environmentally responsive for regenerative medicine. Exp Mol Med. 2013;45:e54. https://doi.org/10.1038/emm.2013.94.
2. Li H, Zimmerlin L, Marra KG, Donnenberg VS, Donnenberg AD, Rubin JP. Adipogenic potential of adipose stem cell subpopulations. Plast Reconstr Surg. 2011;128(3):663–72. Epub 2011/05/17.
3. Han J, Koh YJ, Moon HR, Ryoo HG, Cho CH, Kim I, Koh GY. Adipose tissue is an extramedullary reservoir for functional hematopoietic stem and progenitor cells. Blood. 2004;115(5):957–64.
4. Bourin P, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15:641–8.
5. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, Pasqualini R, Johnstone BH, March KL. A population of CD-34 positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. Circ Res. 2008;102(1):77–85.
6. Gimble, et al. Adipose derived stem cells for regenerative medicine. Circ Res. 2007;100:1249–60.
7. Caplan AI. Mesenchymal stem cells. J Orthop Res. 1991;9:641–50.
8. Caplan A, Mesenchymal DJE. Stem cells as trophic mediators. J Cell Biochem. 2006;98:1076–84.
9. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. Pain Physician. 2008;11(3):343–53.
10. Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, Farjad R, BaghabanEslaminejad M. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. Arch Iranian Med. 2012;15(7):422–8.
11. Jo CH, Yg L, Shin WH, Kim H, Chai JW, Jeong EC, Kim EC, Kim JE, Shim H, Shin JS, Shin IS, Ra JC, Oh S, Yoon KS. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a proof-of-concept clinical trial. Stem Cells. 2014;32(5):1254–66.
12. Longo UG, Papapietro N, Petrillo S, Franceschetti E, Maffulli N, Denaro V. Mesenchymal stem cell for prevention and management of intervertebral disc degeneration. Stem Cell Int. 2012; https://doi.org/10.1155/2012/921053.
13. Sivakamansundari V, Lufkin T. Stemming the degeneration: IVD stem cells and regenerative therapy for degenerative disc disease. Adv Stem Cells. 2013; https://doi.org/10.5171/2013.724.
14. Hohaus C, Ganey TM, Minkus Y, Meisel HJ. Cell transplantation in lumbar spine disc degeneration disease. Eur Spine J. 2008;17(suppl 4):492.
15. Hiyama A, Mochida J, Iwashina T, Omi H, Watanabe T, Serigano K, Tamura F, Sakai D. Tranplantation of Mesenchymal stem cells in a canine disc degeneration model. J Orthop Res. 2008;26:589.
16. Henriksson HB, et al. Transplantation of human mesenchymal stem cells into intervertebral discs in a xenogeneic porcine model. Spine. 2009;34:141.
17. Sakai D, et al. Differentiation of mesenchymal stem cells transplanted to a rabbit degenerative disc model: potential and limitations for stem cell therapy in disc regeneration. Spine. 2005;30:2379.
18. Orozco L, et al. Intervertebral disc repair by autologous mesenchymal bone marrow cells: 2 pilot study. Transplantation. 2011;92(7):822–8.
19. Pettine KA, Murphy MB, Suzuki RK, Sand TT. Percutaneous injection of autologous bone marrow concentrate cells significantly reduces lumbar discogenic pain through 12 months. Stem Cells. 2015;33(1):146–56.
20. Mizuno H, Tobita M, Uysal AC. Concise review: adipose derived stem cells as a novel tool for future regenerative medicine. Stem Cells. 2012;30(5):804–10.
21. Folgiero V, Migliano E, Tedesco M, Iacovelli S, Bon G, Torre ML, Sacchi A, Marazzi M, Falcioni R. Purification and characterization of adipose derived stem cells from patients with lipoaspirate transplantation. Cell Transplant. 2010;19(10):1225–35.

22. Bailey AM, Kapur S, Katz AJ. Characterization of adipose derived stem cells: an update. Curr Stem Cell Res Ther. 2010;5(2):95–102.
23. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell. 2012;9:11–5.
24. FDA Draft Guidance (December, 2014). Minimal manipulation of human cells, tissues and cellular and tissue-based products.
25. FDA Draft Guidance for Industry (October, 2014). Same surgical procedure exemption under 21 CFR 1271.15(b): Questions and Answers Regarding the Scope of the Exception. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm419911.htm.
26. FDA Draft Guidance for Industry and Drug Administration Staff (Nov 2017/Corrected Dec 2017). Regulatory considerations for human cells, tissue, and tissue based products minimal manipulation and homologous use. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research.
27. FDA Draft Guidance for Industry (Nov 2017). Evaluation of devices used with regenerative medicine advanced therapies. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research.
28. FDA Draft Guidance for Industry (Nov 2017). Expedited programs for regenerative medicine therapies for serious conditions. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research.
29. 21st Century Cures Act. US H.R. 6 114th congress, 2016. https://www.fda.gov/RegulatoryInformation/LawsEnforcedbyFDA/SignificantAmendmentstotheFDCAct/21stCenturyCuresAct/
30. Buschmann J, Gao S, Harter L, et al. Yield and proliferation rate of adipose-derived stromal cells as a function of age, body mass index and harvest site-increasing the yield by use of adherent and supernatant fractions. Cytotherapy. 2013;15(9):1098–105.
31. Jurgens WJ, Oedayrajsingh-Varma MJ, Helder MN, et al. Effect of tissue-harvest site on yield of stem cells derived from adipose tissue: implications for cell-based therapies. Cell Tissue Res. 2008;332:415–26.
32. Vilaboa SD, Navarro-Palou M, Llull R. Age influence on stromal vascular fraction cell yield obtained from human lipoaspirates. Cytotherapy. 2014;12:1092–7.
33. Hernigou P, Homma Y, Flouzat-Lachaniette CH, Poingnard A, Chevailler N, Rouard H. Cancer risk is not increased in patients treated for orthopedic diseases with autologous bone marrow cell concentrate. J Bone Joint Surg Am. 2013;95:2215–21.
34. Yshiya S, Dhawan A. Cartilage repair techniques in the knee: stem cell therapies. Curr Rev Musculoskelet Med. 2015:457–66.
35. Alaseem AM, Madiraju P, Aldebeyan SA, Noorwali, H, Antoniou J, Mwale F. Naproxen induces type X collagen expression in human bone marrow derived mesenchymal stem cells through up-regulation of 5-Lipoxygenase. Tissue Eng: Part A. 2015;21(1 and 2).
36. Fredriksson M, Li Y, Stalman A, Haldosen LA, Fellander-Tsai L. Doclofenac and triamcinolone acetonide impair tenocytic differentiation and promote adipocytic differentiation of mesenchymal stem cells. J Orthopaed Surg Res. 2013;8:30.
37. Halme DG, Kessler DA. FDA regulation of stem cell based therapies. N Engl J Med. 2006;355:1730–5.
38. Fournier PF. Reduction syringe lipo contouring. Dermatol Clin. 1990;8:539.
39. Hunsted JP. Tumescent and syringe liposculpture: a logical partnership. Aesth Plast Surg. 1995;19:321–33.
40. Housman TS, et al. The safety of liposuction: results of national survey. Dermatol Surg. 2002;08:971–8.
41. Klein J. Tumescent technique chronicles. Dermatol Surg. 1995;21:449–57.
42. Aronmitz JA, Lockhart RA, Hakakian CS. Mechanical verses enzymatic isolation of stromal vascular fraction cells from adipose tissue. Springerplus. 2005;4:713.