Regenerative Medicine

Technical Issues in Harvesting and Concentrating Stem Cells
(Bone Marrow and Adipose)

Jay E. Bowen, DO

Abstract

The area of regenerative medicine, through the use of cell-based or biologic therapies, affords various options even with the choice of autologous stem cells and requires the clinician to use the currently evolving science along with the art of medicine. Potential sources for stem cells are embryos, fetuses, and adults (adipose tissue or adult cells, usually from skin [induced pluripotent], amniotic fluid, cartilage, bone marrow, menstrual blood, peripheral blood, placenta, skin, teeth, synovium, or umbilical cord blood). Issues regarding the source of stem cells include ethical and political/regulatory concerns, cell frequency, ease/difficulty of extraction, ease/difficulty of graft preparation, and host reaction to the implant. The most common sources of stem cells for orthopedic conditions are adipose tissue and bone marrow. Various opinions are expressed within the medical literature for and against both of these stem cell sources. This article will review patient concerns and preparation for obtaining cells; equipment, supplies, and staffing for the procedures; techniques, pearls, pitfalls, and risks of the procedures; and the manner of/options for processing the cells.

Introduction

Consideration of cell therapy for musculoskeletal conditions encompasses ethical and regulatory issues, donor site choice (bone marrow versus adipose tissue), and technical aspects, as well as potential risks of obtaining cells from the source.

Use of autologous bone marrow or adipose cells enables the avoidance of ethical concerns and graft versus host reactions. Regulatory issues are minimal if the cells are less than minimally manipulated and if no cell expansion occurs. Bone marrow aspirate contains relatively fewer absolute numbers of cells compared with adipose tissue [1-8]. Debate exists regarding whether the stem cell source or number is of greater importance. Another issue that has been raised is the manner of obtaining the aspirates—that is, the use of local anesthetic agents and resultant cell viability, necrosis, or apoptosis [9-11].

Potential adverse effects of aspiration include systemic infection, local infection at the donor site, and severe anemia, whereas contraindications of placement include infection at the graft site, blood dyscrasias, and past malignancy [7].

Bone marrow aspiration (BMA), when performed appropriately, results in limited pain, is relatively safe, and can be completed in short period. Careful procurement is necessary with adipose tissue to maximize viability, but the procedure is safe and can be completed in a similar period as BMA. Because stem cells are difficult to separate from the adipose tissue structure, digestion methods have been described for adipose tissue to obtain the cells, but these methods are not compliant with the current Food and Drug Administration (FDA) guidelines regarding minimal manipulation [12]. The complexity of graft preparation is similar for bone marrow and adipose aspirates.

Although differentiation into various tissue types (eg, cartilage) is believed to be an important characteristic of stem cells, additional important effects of these cells also have been described, including paracrine effects, angiogenesis potential, proliferation ability, resistance to apoptosis, differentiation potential, and potential to enhance repair [1-7]. Current research is providing guidance in this rapidly evolving field with regard to determining the best way to employ various stem cell sources to harness these various effects to increase the effectiveness of cellular therapy treatment.
Consents, Preprocedure Patient Preparation, and Testing

As with any procedure, informed consent is mandatory. Informed consent is not merely a signature on a page but a discussion at the time the decision is made to perform the primary procedure and again prior to the actual procedure so the individual has time to ask questions, understand and ponder the implications of the procedure, and be comfortable with the potential risks. Explaining the procedure to the patient also can reduce anxiety. The discussion must be between the physician (not a designee) and the patient. The consent should be written and include the following basic information: the patient’s name, the date and time, the institution, the name of the procedure, the name of all the practitioners involved in the procedure, risks, benefits, alternative procedures and treatment, a statement that the procedure was explained, a statement that the patient had the opportunity to ask questions, the signatures of the patient or a guardian and of a witness, and the name of the person who explained the procedure [13,14]. Given the lack of controlled studies for stem cell therapies, informed consent should include a statement that the treatment agreed upon is not the standard of care and is considered experimental in nature at this time.

The greatest risks of these procedures are infection and hematoma. Because a large-bore needle is used to penetrate the skin, inclusion of a stylet may reduce the infection risk. In patients with a history of osteoporosis, there is a greater risk of traversing the ilium into the pelvic cavity or of fracture during BMA. With regard to the adipose procedure, the occurrence of local deformities is an additional potential risk. Additional details of the risks are provided in the sections on bone marrow and low-pressure liposaspirate technique.

Once it has been determined that the procedure should be performed and patient consent has been obtained, procedural planning is necessary. A review of the patient’s medications may reveal use of pharmaceutical drugs and supplements that could have an adverse effect on healing. Use of nonsteroidal anti-inflammatory medications should be stopped at least 5 days prior to the procedure because of the theoretical and known negative impact on healing [15]. Corticosteroids have been found to have a negative impact on tissues even with a one-time injection or with use of inhaled steroids for asthma, even though it was once thought they were not systemic [16,17]. The procedure should be delayed for at least 8 weeks after a steroid injection and use of inhaled steroids should be discontinued, if possible, prior to the procedure. Fluoroquinolones have a black box warning regarding the risk of tendon rupture (according to package information provided by pharmaceutical companies). The potential negative impact on soft tissue should be taken into account during the treatment process. You should remind the patient to contact you before taking any new medication or supplement in the postprocedure period so you can discuss options with the patient in case of a concern. Concerns have been raised regarding the effect of proton pump inhibitors (omeprazole) and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (eg, atorvastatin [Lipitor]) on in vitro stem cell growth. HMG-CoA reductase inhibitors do inhibit angiogenesis [18]. For persons who are or have been taking an HMG-CoA reductase inhibitor, supplementation with coenzyme Q-10 should be considered [19].

Optimal laboratory testing has not been determined. However, a baseline hemoglobin and hematocrit should be considered if harvest of a high volume of aspirate is planned. Various deficiencies can adversely affect tissue healing, which is the goal of a regenerative procedure, and thus screening should include at least 25-hydroxy vitamin D, albumin, zinc, and hormones (free T3 with a goal of a high to normal level, free testosterone, and estradiol [E2] and progesterone in postmenopausal women) [20-22]. Most procedures should be performed with use of a local anesthetic with a complete understanding of the toxicity levels of such anesthetics (Tables 1 and 2). For example, a maximum dose of lidocaine is 4.5 mg/kg, up to 300 mg lidocaine without epinephrine, or 7 mg/kg, up to 500 mg lidocaine with epinephrine. Depending on the referenced toxic levels chosen (ie, Medscape [23] or Goldfrank et al [24]), the maximum recommended volume would be between 32-45 mL of 1% lidocaine without epinephrine in a man weighing 70 kg.

Specific effects regarding stem cells include their impact on the tissues being treated and the stem cells themselves. Toxicity has been demonstrated to the tenofibroblast, chondrocyte, adipose-derived stem cell (ASC), and human mesenchymal stem cell, as well as to rabbit and bovine intervertebral disk cells [9,25-39]. Toxicity can be a concern when obtaining a liposaspirate because as little as 0.03% of lidocaine can have a negative effect on ASCs [32]. When obtaining cells from the bone marrow, toxicity is not an issue.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Toxic dosages of various local anesthetics</th>
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<tr>
<td>Agent</td>
<td>Minimum Toxic Dose (mg/kg)</td>
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<tr>
<td>Procaine</td>
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<tr>
<td>Tetracaine</td>
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<tr>
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<td>22.8</td>
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<td>Bupivacaine</td>
<td>1.6</td>
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<tr>
<td>Etidocaine</td>
<td>3.4</td>
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</table>

because the anesthetic is outside the marrow cavity, but care should be taken with the lipoaspirate as described in the technique. Anesthetics can affect proliferation and can have other negative effects if residual anesthetic is present when the graft is placed. When the graft is placed, anesthetic should only be used outside the desired treatment region and/or a regional nerve block should be used to make the procedure as comfortable as possible. Variability exists in the tissue toxicity based on the type of anesthetic, its concentration, and the duration of exposure. Overall, ropivacaine appears to be the least toxic anesthetic.

Pain Control and Issues Regarding Local Anesthetics

The first step in addressing any procedure-related pain is to reduce anxiety by explaining the procedure and reassuring the patient that you will inform him or her about what is happening each step of the way. Some patients will ask to be “knocked out,” but this step is unnecessary; furthermore, use of a general anesthesia increases the risk and cost, and many patients have adverse effects from the anesthesia. Reassuring patients that the procedure is well tolerated by others can be comforting. In rare cases a single oral dose of an anxiolytic (e.g., diazepam) can be provided to the patient 45 minutes prior to the procedure. If need be, an oral analgesic can also be given. Some patients choose to wear ear buds and listen to calming or distracting music.

"Liberal" use of the local anesthetic is essential, first with a small needle skin wheel and then at the peristeum for BMA. This approach generally eliminates the need for oral preprocedure pain medication. With a lipoaspirate, a triangular field block is performed and the addition of epinephrine minimizes bleeding and postprocedure pain. The addition of sodium bicarbonate reduces the burning sensation and increases the onset of action (increased pH/more alkaline increases the nonionized component and transfer across the cell membrane). Because local anesthetics can have a negative effect on stem cells, consideration of an oral analgesic (opiate) 45-60 minutes prior to the liposaspirate procedure can be considered.

Bone Marrow

Equipment, Supplies, and Staff

Specialized equipment necessary for BMA includes an imager (either ultrasound or fluoroscopy) and a disposable aspiration device (a T-handle BMA needle [e.g., Jamshidi, CareFusion Corp, San Diego, CA] or a drill [e.g., Arrow OnControl device, Teleflex, Shavano Park, TX]). If a manual aspiration is planned, an 11-gauge trephine needle is recommended to avoid device failure with the axial loading and limit local tissue disruption to minimize morbidity. The needles have either a slanted or a diamond tip. Compared with a slanted tip, the diamond tip has less of a tendency to slide, and its additional edges results in easier bone entry. The drill reduces the axial force needed, and when a drill is used, a smaller gauge needle (15 gauge) can be used, which reduces the morbidity and pain [40]. The drill requires use of less force, reducing stress on the physician’s hand/wrist. Use of less force results in dramatically fewer episodes of sliding along the iliac

<table>
<thead>
<tr>
<th></th>
<th>Finland</th>
<th>Germany</th>
<th>Japan</th>
<th>Sweden</th>
<th>United States</th>
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<tr>
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<td></td>
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<td>150 mg</td>
<td>100 mg (epidural)</td>
<td>150 mg</td>
<td>175 mg</td>
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<td>150 mg</td>
<td>225 mg</td>
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<td>500 mg</td>
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<td>350 mg</td>
<td>550 mg</td>
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<tr>
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<td>No mention</td>
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<td>225 mg</td>
<td>225 mg (300 mg*)</td>
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* For brachial plexus block in adults.
crest before engaging. Additionally, use of a drill significantly reduces procedure time [40-42].

Oxygen, pulse oximetry, a crash cart/box, and an automatic electronic defibrillator should be present. In addition, standard procedure supplies for an aseptic procedure are necessary. An antiseptic solution to clean the patient’s skin must be used to minimize the risk for infection. A chlorhexidine-alcohol solution (2% chlorhexidine gluconate and 70% isopropyl alcohol) has been shown to be more effective than povidone-iodine (Betadine) [43]. It is important to remember that effective antibacterial effects occur after the agent has dried. Standard sterile technique is used throughout the procedure, including the use of a face mask, caps, sterile gloves, and drapes or towels. If ultrasound guidance is being used, a sterile transducer condom can be applied. A standard procedure support tray should include an 18-gauge needle to draw up medication, a 27-gauge × 1.25-inch needle for a skin wheal, a 22-gauge × 2.75-inch needle for anesthetizing the deeper tissue and periosteum, a 10-mL syringe, syringes for bone marrow (30 mL × 2), Luer lock syringe caps, sterile gloves, gauze, a needle stick pad, and a bag(s) to transport the bone marrow sample. Additional supplies include ice packs, sterile 2 × 2 gauze squares, and a transparent occlusive dressing cover (eg, Tegaderm, 3M, St Paul, MN).

Medications include 1% lidocaine without epinephrine, 50% dextrose, sodium bicarbonate, normal saline solution, and heparin (20,000 units/mL × 2 and 10,000 IU/mL × 1). Additionally, a 1000 IU/mL solution of heparin should be made to rinse the aspirating trocar and syringes prior to initiating the procedure.

Staffing depends on whether ultrasound or fluoroscopic guidance is used and the specifics of the procedure room. An assistant is needed to transfer items to the procedure field in a sterile manner and to assist with positioning or controls on the imaging device (ultrasound or fluoroscopy). An additional assistant can enhance the ease of the procedure and improve efficiency, but having an additional assistant is not mandatory.

Use of headphones or the patient’s choice of music are also options to consider.

**BMA Technique**

The patient should be prone for the BMA procedure. Oxygen (1-1.5 L) can be administered via a nasal cannula for patients who feel they cannot breathe efficiently with their nose and mouth in a pillow or table cutout. Having pulse oximetry in place throughout the procedure is recommended. Placement of pillows under the abdomen should be avoided because a firm surface is required, especially when performing the manual technique. A pillow or roll can be placed under the ankles to reduce lumbar stress. When fluoroscopy is used, an initial lumbosacral scout view will provide a reference followed by a 20° ipsilateral oblique and caudal tilt to visualize the ilium, iliac crest, and posterior superior iliac spine (PSIS). A radiopaque marker such as an 18-gauge needle on the skin can be used to identify 1 cm lateral to the PSIS. When the needle is inserted, a hub view will be obtained to the desired location (Figure 1).

With ultrasound guidance, use of a linear transducer is initially attempted unless the size of the patient requires use of a curvilinear transducer. The PSIS is identified with the medial aspect of the probe fixed on it while the lateral portion of the probe is swept from pointing to the greater trochanter to the anterior superior iliac spine. Within this arc the aspirate needle can be guided in plane from lateral to medial to the ilium about 1-2 cm from the iliac crest surface for marrow entry and then can be slide cranial for additional sites (within zone 1 as shown in Figure 2) [44].

Different BMA needle tips are available. Compared with other BMA needle tips, the diamond tip has less of a tendency to slip on the bone’s surface prior to engaging. Use of a 180° rotating motion (alternating 90° left and 90° right) with firm pressure will allow the tip to enter the bone; use of excessive axial force is unnecessary. An alternative approach is to have one person hold the needle in place against the bone while a second person taps the aspirating needle with a mallet. When entering the bone marrow cavity using a manual technique, a loss of resistance will occur in most cases. If the trocar has been advanced a few millimeters without reduced resistance, then stopping to test for marrow aspiration should be considered.

If a rotatory device/drill is used to enter the marrow cavity, the complementary needle will have depth markings. Once the cavity is reached, one will observe the needle line markings (Figure 3) advance quickly and the drill will speed up, resulting in faster needle rotation, which causes a sound with a higher pitch.

![Figure 1. A hub view to the desired location (arrow = hub view of the aspirating needle). PSIS = posterior superior iliac spine.](image-url)
The goal of BMA for regenerative treatments is to obtain the greatest number of nucleated cells. The cells are adherent to the trabeculae, so much of the aspiration is peripheral blood. Cells need to be harvested from multiple sites to maximize the number of cells aspirated. Muschler et al [45] noted 2-mL aspiration from each site, although other practitioners aspirate 4 mL [46]; however, practically, 5-10 mL per site is acceptable. Generally in the parallel technique between the bone tables, the needle is rotated and advanced between collection of aspiration volumes. Some Jamshidi needles have additional side ports that may be used to obtain cells from different areas of the marrow. Once the marrow cavity is entered and aspiration is initiated, a deep pelvic discomfort or mild to moderate pain or ache may be felt. A slower rate of aspiration and slower removal/replacing of the stylet may minimize this discomfort.

With the entry site described, 4 separate sites can be entered while maintaining the tip subcutaneously without removing the needle from the skin (Figure 4A, B).

The trajectory of the needle described is relatively perpendicular to the ilium, toward the thicker edge, offering some degree of safety. If the needle easily passes through the bone into the marrow cavity, the other table on the ilium will temporarily stop the needle from entering the pelvic cavity (Figure 5). This situation is only good if one does not continue to drive the needle further and the position is checked. This technique is different from the aspiration parallel [41] to the iliac wing tables (Figure 6), which increases the risk of neural and vascular injury [44,47-48].

A greater risk of losing bone contact occurs with the parallel method, and when performing the manual technique, the force required may cause the trocar to enter the abdominal cavity, with the potential for infection or visceral injury. If the needle goes into a lateral and posterior position, the superior cluneal nerve or superior gluteal vessels can be injured (Figure 7). A vascular injury may lead to an enlarging hematoma (external iliac artery or superior/inferior gluteal arteries). Other reported complications are fracture, sciatic nerve palsy, lateral femoral cutaneous injury, soft tissue injury, sacroiliac joint lesion, and postprocedure pain [44]. Hernigou et al [44] noted only 8 complications in 1800 marrow aspiration procedures, which is reassuring. However, an additional study by Hernigou [47] noted 410 cadaveric trocar entries (parallel in various sectors) with 114 medial or lateral table breaches (28%), which is very concerning. There is a greater risk of complications if the surgeon is inexperienced or the patient is obese (body mass index >30).

The preferred technique (perpendicular) appears to be the most common technique used by hematologists. Hematologists treat a different population than that treated in a musculoskeletal practice, and persons treated by hematologists may have a greater tendency for bleeding. Bain [49] reported biopsy morbidity and mortality but did not detail the technique or site. Of 58,596 procedures, there were 26 complications (14 hemorrhages, 7 needle-related incidences, 3 infections, and 2 miscellaneous complications).

**Postprocedure Care**

After the trocar is removed, pressure is applied to minimize bleeding. Once hemostasis is obtained, gauze and a clear, occlusive dressing are applied and the patient is placed in the supine position with ice applied to the area for 10-15 minutes. Immediate icing reduces the development of swelling/bruising and allows the patient to be monitored. After 10-15 minutes of observation, repeat vital signs should be obtained, and if the patient is stable, he or she is discharged with a companion. The individual is encouraged to apply ice to the donor site for 15-20 minutes each hour 3-5 times or more often if pain returns. The area should be kept clean and dry for 12 hours with no soaking in a hot tub, pool, or bath for 3 days. Written instructions assist in achieving compliance. The area may be sore for several days to a week, but soreness should not be increasing. Increased donor site pain, discharge, erythema, chills/sweating, or fever should prompt the patient to return for a re-evaluation to inspect the area for infection, a hematoma, or other
problems. Rehydration by drinking plenty of water for 24 hours after the procedure should be encouraged, and strenuous activity or heavy lifting should be avoided for 5-6 hours.

Low-Pressure Lipoaspiration

Equipment, Supplies, and Staff

When initially performing lipoaspiration, ultrasound guidance can be very advantageous, but once one’s skill level improves, it is not always needed. However, it is definitively helpful in thin persons. A lipoaspirate needle is needed (eg, a needle manufactured by Tulip Medical Products, San Diego, CA), along with some type of syringe-locking device (eg, Snap Lok or Johnnie Lok, Tulip Medical Products). It is important to know which syringe the locking device is designed to fit, because some locking device will not fit syringes universally. Lipoaspirate cannulas can be 1.67-2.4 mm in diameter and of varying lengths (8-20 cm). A recommendation is 2.1 mm × 10 cm. Consider cell-friendly equipment, because the viability of cells is important for reimplantation. Also consider the ease of cleaning the cannula and autoclaving versus newer options of disposable cannulas.

Oxygen, pulse oximetry, a crash cart/box, and an automatic electronic defibrillator should be present. The other necessary supplies are similar to those used for BMA: a chlorhexidine-alcohol solution (2% chlorhexidine gluconate and 70% isopropyl alcohol), sterile drapes or towels, a sterile ultrasound transducer condom, a standard procedure support tray with an 18-gauge needle, a 27-gauge × 1.25-inch needle, a 22-gauge × 2.75-inch or 22-gauge × 4-inch needle for anesthetizing the harvest field, gauze, a No. 11 blade, a 20-mL syringe (compatible with the locking device as previously noted), and sterile gloves.

Medications include 1% lidocaine with epinephrine (1:100,000), sodium bicarbonate, normal saline solution, and anticoagulant citrate dextrose solution—formula A, United States Pharmacopeia (ACD-A). Also needed are ice packs, sterile 2 × 2 gauze squares, and a transparent occlusive dressing cover (eg, Tegaderm). Use of an anesthetic with epinephrine will reduce the amount of local bleeding, which is also beneficial for speeding recovery.

An assistant is needed to transfer items to the procedure field in a sterile manner and assist with positioning or controls on the imaging device, if such a device is used.

Low-Pressure Lipoaspiration Technique

Approximately 400,000 liposuction surgeries are performed in the United States each year, and these procedures yield anywhere from 100 mL to >3 L of lipoaspirate tissue [50]. The desired volume for regenerative medicine is significantly lower—usually less than 30 mL.

If a patient is not too thin, the easiest sites to obtain lipoaspirate are over the gluteal area or umbilical

Figure 4. (A) The osseous entry site in the pelvis for bone marrow aspiration needle via a “perpendicular” approach. The blue arrow represents the needle for aspiration. (B) The black box represents the footprint of the ultrasound probe. The blue arrow represents the needle for aspiration.

Figure 5. A perpendicular approach to the marrow cavity and two tables of the ilium. The second table provides some measure of prevention of inadvertently entering the pelvic cavity.
The patient is placed prone and pillows can be used for comfort if desired. Alternative sites for the donor site can be the lower abdomen, medial or lateral thigh, trochanteric region, and flank. If the flank is considered site, the lateral decubitus position is preferred, and use of a curved cannula can be contemplated. Use of ultrasound in thin patients can help determine the thickness of adipose in the aspiration site considered and the optimal position to prepare for the procedure. Ultrasound assistance may be considered for the initial learning curve or in thin patients.

Once the site has been determined, it should be marked and the area should be cleansed with a chlorhexidine-alcohol solution and draped in the usual manner. If the procedure is being performed at the same time as the BMA, the same puncture site lateral to the PSIS can be used as the starting point. A solution of 2 mL of 1% lidocaine with epinephrine and 8 mL of normal saline solution is made. Inclusion of epinephrine will reduce local bleeding. Consideration of ropivacaine and the least degree of anesthetic infiltration necessary should be considered to maximize the cell viability of the aspirate. A 22-gauge × 4-inch needle is used to infiltrate the region from medial to lateral in a fanlike distribution toward the iliac crest and greater trochanter (Figure 8, red lines).

An alternative anesthetic technique may not provide the same degree of comfort but may reduce the negative impact of the local anesthetic on the aspirate and future graft. This technique involves infiltrating the margins of the field (Figure 8, blue lines) and focusing extraction from the center of the field, away from the location of the anesthetic.

To perform the procedure, place 2 mL of ACD-A in the aspiration syringe and attach the cannula and locking device. If a cannula was chosen with ports on only one side, line up the locking device as a marker to identify the ports once the cannula is in place. Make a small skin nick at the initial anesthetic injection site with a No. 11 blade to allow the blunt tip aspiration cannula easier access through the skin. Insert the cannula into the field and make a few passes to distribute the anesthetic and begin to break down/mobilize the adipose tissue. Begin by making sure the aspiration holes are through the skin.
pull back on the syringe plunger, and lock it in place. The practitioner can then freely move the cannula back and forth in the anesthetized region. If the cannula has ports only on one side, they can be directed laterally, especially in a thin individual, to minimize trauma to the underlying muscle or aspirating too much volume close to the skin, which can result in skin dimpling. Pinching the tissue to raise it up often makes the process easier. Additionally, pinching the tissue can stimulate mechanoreceptors and reduce procedural pain. The initial passes will be more difficult and return will be more limited, but later passes as the tissue is mobilized will be easier and smoother. The aspirate will appear yellow, preferably with limited blood, and oily. If vacuum is lost, remove the cannula, “unlock” the plunger, hold a sterile gauze over the cannula holes, express the excess air from the syringe, and repeat the initial steps [51-53]. The usual goal is 12-20 mL of liquefied adipose. If a larger volume is desired, use of a second site can be considered.

With the advent of microcannula, cell-friendly equipment, and a low-volume technique, few complications occur. A patient may be intolerant to the addition of epinephrine, with increased anxiety, which must be weighed against the bleeding/bruising/postprocedure pain. This procedure harvests a relatively low volume of aspirate, and therefore skin dimpling is uncommon. Maione et al [54] reviewed 1000 cosmetic surgery cases for postprocedure complications with a mean aspiration volume of 68 mL, which is significantly greater than that proposed for the current procedure. There were 85 donor site complications (2 hematomas and 83 local deformities) and 5 recipient site complications (4 infections and 1 graft rupture).

**Postprocedure Care**

When the cannula is removed, pressure is applied to the insertion site and the area is cleansed. A 2 x 2 gauze square with or without antibiotic gel and a clear occlusive bandage (eg, Tegaderm) are applied. Ice is applied to the donor site to minimize bleeding and pain and to speed recovery. Close attention and the time of monitoring should be adhered to because the skin was anesthetized. After 10-15 minutes of observation, repeat vital signs should be obtained, and if the patient is stable, he or she is discharged with a companion. Icing will reduce swelling, but the patient must understand that ice should not be applied for more than 20 minutes in the immediate time after the procedure because of the use of a local anesthetic during the procedure. The area should be kept clean and dry for 24 hours with no soaking in a hot tub, pool, or bath for 3 days. Written instructions assist in achieving compliance because the patient can refer to them. The area may be sore for several days to a week, but the soreness should not be increasing. Increased donor site pain, discharge, erythema, chills/sweating, or fever should prompt the patient to return so the area can be inspected for infection. Rehydration by drinking plenty of water for 24 hours after the procedure should be encouraged, and strenuous activity or heavy lifting should be avoided for 5-6 hours.

**Concentrating**

In the United States, the FDA has determined that more than minimal manipulation of a graft/cells is considered a new drug and under their purview. Minimal manipulation would be under the jurisdiction of each State’s Board of Medical Examiners as the practice of medicine. Some treatments are rendered under a research protocol with institutional review board monitoring. This information is not meant to be an exhaustive medical/legal briefing but a caution to be fully informed regarding whatever process is undertaken. These issues are discussed in more detail in another section of this supplement. Additionally, do not rely on a commercial company or representative’s advice without verification.

Processing of the initial aspirate is necessary to remove red blood cells (RBCs) that can interfere with healing and cause increased procedural pain, reduce total volume, and concentrate the rare-occurring cells that will produce the desired healing effect. The final product should be of a volume sufficient to address the targeted disease with the maximum amount of multi-potent cells and growth factors.

**Commercial Kits**

A variety of commercially available kits present in the market can be considered for point of care treatment. Some kits include only the equipment for aspiration, and others include both aspiration and processing equipment. The companies that produce kits for BMA include but are not limited to Arthrofix, Biomet, Cellding Biosciences, Alliance Spine (Cyclone), EmCyte Corp (GenesisCS), Harvest Technologies Corp, MAGELLAN, Globus Medical (RETRIEVE BMA), and Synthes (chronOS). Companies that produce products to harvest adipose tissue include AdiStem, Puregraft, and Klinik Solutions (Lipogems). Few to no human studies have been conducted to demonstrate the effectiveness of these systems for orthopedic conditions, and certainly no studies have demonstrated the superiority of one system over another.

**Independent Performance/Supplies**

In lieu of using a commercial product, the concentration of the obtained aspirates can usually be performed in a less expensive manner with the ability to “tailor” the product with experience. The process should be performed in a closed system fashion and with use of a bio-safety cabinet, if one is available. General
supplies may include syringes, needles, syringe caps, test tubes and rack(s), conical tubes and caps, sterile bags for transport of the aspirated graft, a marking pen for plastic, normal saline solution and/or phosphate-buffered saline solution, sterile gloves, cleansing solutions (eg, CaviCide [Metrex] and Chloraprep [Carefusion]), centrifuge, and micropipette and its supplies. A dedicated area should be allocated for the setup of processing of the aspirate to ensure sterility and efficiency. Additional supplies can be added or subtracted depending on the method chosen or changes desired. If quantification is considered, then investing in a cell counter is necessary.

**Bone Marrow**

Connolly and Shindell [55] first used bone marrow aspirate to assist in orthopedic treatment in 1986 and went on to popularize its use [56-60]. Wakitani et al [61] expanded mesenchymal stem cells (MSCs) from bone marrow for the treatment of knee osteoarthrosis as early as 2002, with others simply concentrating the aspirate for treatment. In 2008, Sakai et al [62] detailed an inexpensive “manual blood bag” process. The aspirate from each syringe was added to a bag and then gravity filtered. The bag is centrifuged at 1200g, with the resultant bottom layer being RBCs, which can be drained. A second spin at 3870g results in the plasma being on top so it can be aspirated and discarded, leaving a small amount of RBCs at the bottom layer, the desired buffy coat in the middle, and some plasma for resuspension on top. The problem with this method is that too many RBCs are left in the final product, and a centrifuge that can handle a blood bag is needed.

A modification of the process developed by Sakai et al can avoid use of a bag and minimize the inclusion of RBCs. The BMA from the syringes is added to conical tubes and centrifugation is performed. The tubes are first spun for 10 minutes at 1200g, which will separate the RBCs and plasma. The RBCs are removed via pipette, and a second high-speed spin is performed at 3870g for 7 minutes. The Buffy coat from each tube is pipetted into a combined tube and some plasma is added for the desired final volume. This substance is then placed into a syringe that will be used for the therapeutic procedure.

Many investigators report revolutions per minute (RPMs) when discussing sample processing. One must remember that G force is consistent among centrifuges, but RPMs vary based on the radius of the centrifuge. The difference is usually not significant in this process. Various centrifugation spin rates have been proposed by commercial companies, which can be considered when processing the aspiration independently (Table 3) [63,64].

**Adipose Tissue**

Because only minimal manipulation of adipose tissue may be performed to maintain compliance with current FDA regulations, only minimal manipulation techniques will be described here.

The first component is the material obtained from the lipoaspirate [65,66]. The easiest and most basic method is to allow natural gravity separation for about 10 minutes and then discard the oil component. The oily component is believed to be “inflammatory” and does not contain “stem cells” [67]. Add 10 mL of normal saline solution and mix in a closed syringe-to-syringe setup (Luer-Lok connection; Figure 9). Place the mixture in a centrifuge tube and spin for 3.5 minutes at 1600 rpm. Remove the RBC layer. The graft will be too viscous to be injected through an 18- to 22-gauge needle. A mechanical emulsifier can be interconnected between syringes with a back and forth/push-pull process to facilitate flow through a smaller gauge needle. Be sure to test the ability to administer through the desired needle gauge prior to the procedure. The needle can become clogged, so it is important to have additional supplies available at the time of the therapeutic procedure.

Raposio et al [68] proposed a novel technique for isolating adipose-derived stem cells. The aspirate, in

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**Table 3**

<table>
<thead>
<tr>
<th>Company/Product</th>
<th>Volume</th>
<th>Spin No. 1 × Time</th>
<th>Spin No. 2 × Time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>60 mL</td>
<td>3600 rpm × 10 min</td>
<td>N/A</td>
</tr>
<tr>
<td>PureBMC</td>
<td>75 mL</td>
<td>3800 rpm × 2.5 min</td>
<td>3800 rpm × 5 min</td>
</tr>
<tr>
<td>Arteriocyte</td>
<td>30-60 mL</td>
<td>2800 (610g) × 4 min</td>
<td>3800 rpm (1240g) × 6 min</td>
</tr>
<tr>
<td>Celling Technologies</td>
<td>Not stated</td>
<td>3200 rpm × 12 min</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* The reason for 2 spins is to remove the red blood cells.
tubes, is placed in a vibrating shaker at 6000 vibrations/minute for 6 minutes and then centrifuged for 6 minutes at 1600 rpm. The resultant pellets from each tube were pipetted and placed into a 10-mL syringe. They tested their isolate and determined a 12% increase in stem cell population compared with the standard enzymatic digestion process [66]. Processing time was reduced from 180 minutes to 15 minutes, and there were no animal-derived reagents or collagenase, which carry their own inherit risks and qualifies as being considered more than minimal manipulation.

Conclusion

Regenerative medicine has progressed into exciting new territory with use of biologic and cellular based treatments. Bone marrow and/or adipose sources appear to have the greatest scientific basis for successful treatment outcomes in patients who have orthopedic conditions, especially those involving cartilage disease. We must inform patients of the risks and potential benefits of these new treatment options and provide unambiguous information regarding the current evidence for the use of stem cell treatments. Assessment of the chosen procedure will need to include evaluation of the process and guide future treatment protocols so outcomes will be meaningful and enable future modifications to improve results. With knowledge of the current evidence of potential stem cell sources, potential applications, and regulatory issues, as well as appropriate supplies and proper preparation, the procedures should have little morbidity and be easily accomplished to maximize the potential benefits of these treatments.

References

13. 42 CFR §482.51 (b) (2) Interpretive Guideline A-0392.
23. 42 CFR §482.51 (b) (2) Interpretive Guideline B-0292.

Disclosure

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