Chapter 40
Bone Marrow Derived Stem Cells and Their Application in Pain Medicine

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Bone marrow is increasingly being used to treat musculoskeletal disorders. There is promising early clinical data on the treatment of knee, hip, and shoulder osteoarthritis, as well as intervertebral disc disease [1–10]. The most common type of therapy uses bone marrow concentrate (BMC), which is comprised of the isolation of the buffy coat found within centrifuged bone marrow aspirate [11]. It is considered that the active components within bone marrow are mesenchymal stem cells, but other cells are also present.

Treatment with BMC is allowing for a shift in orthopedic care from surgeries to remove or modify tissue to precise, image-guided injections to help the healing of injured or degenerated tissues. The advantages to this approach are obvious: the ability to reduce the morbidity associated with more invasive surgical procedures, as well as the ability to prompt tissue healing. Finally, the implications for pain management clinicians are also game-changing, as their interventional skill set fits well with these new approaches to orthopedic problems.

40.1 Indications

As of 3 April 2016, a total of 8428 patients had been treated for orthopedic conditions with any type of bone marrow stem cell therapy and had their results (outcomes or adverse events) published and listed in the U.S. Library of Medicine. Table 40.1 lists the disease areas with the most published outcome information for bone marrow concentrate (BMC).

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Table 40.1 Summary of evidence for common orthopedic conditions treated with bone marrow concentrate

Pathology	Total treated, *n*	References
Osteonecrosis	342	[12]
Knee osteoarthritis	1018	[4, 13]
Shoulder rotator cuff	200	[1, 6]
Hip osteoarthritis	201	[17, 18]
Degenerative disc disease	47	[7–9, 19, 20]
Ankle disorders	92	[18, 21]
Epicondylitis	30	[22]

40.1.1 Osteonecrosis

The largest published study is Hernigou et al. (n = 342) [12]. Hips of Association Research Circulation Osseous (ARCO) grade 1 or 2 showed approximately an 80% long-term likelihood of not requiring arthroplasty. With more severe grades (ARCO grades 3 and 4), there was declining success.

40.1.2 Knee Osteoarthritis

Table 40.2 summarizes the major studies, including both BMC and culture-expanded mesenchymal stem cells (MSCs) for the treatment of knee osteoarthritis (OA):

- Published data demonstrate good clinical results. The Vangsness study [13] revealed an increase in meniscus size in one in four patients, and the Vega study [14] showed improvement in cartilage signal on follow-up MRI.
- We have published a large case series demonstrating promising pain and functional outcomes; the addition of a fat graft did not improve outcomes over injecting BMC alone [15].

40.1.3 Shoulder Rotator Cuff

Two published studies support the use of BMC for shoulder OA and rotator cuff tears (Table 40.3):

- Hernigou et al. [6] published a comparison trial of surgical shoulder rotator cuff repair with or without the use of injected BMC. The BMC group had approximately one half the re-tear rate of the surgery-only group.
- The authors have completed a case series of 102 patients with shoulder OA and rotator cuff tears who demonstrated significant reductions in pain and increases in validated functional metrics [1].

Table 40.2 Summary of published research using BMC or culture-expanded bone marrow MSCs for knee osteoarthritis

Study	Study type	Intervention	Patients, *n*	Stem cells used	Functional improvement	Notes
Vangsness et al. [13]	DB RCT	Partial menisctomy with MSC injection	55	Allo cultured bone marrow MSCs	Yes	1 in 4 patients with increased meniscus volume
Centeno et al. [15]	Prospective case series	Image-guided injection	840	BMC	Yes	2/3 of patients were TKA candidates
Kim et al. [16]	Prospective case series	Injection	49	Autologous cultured bone marrow MSCs	Yes	Full-thickness chondral lesions <6 cm/2 responded best
Vega et al. [14]	RCT	Injection of MSCs vs HA	30	Allo cultured bone marrow MSCs	Yes	Improved cartilage signal on MRI T2 mapping

BMC bone marrow concentrate, *DB* double blind, *HA* hyaluronic acid, *MSCs* mesenchymal stem cells, *RCT* randomized, controlled trial, *TKA* total knee arthroplasty

Table 40.3 Summary of published research using BMC for shoulder osteoarthritis and rotator cuff tear

Study	Study type	Intervention	Patients, *n*	Stem cells used	Functional improvement	Notes
Centeno et al. [1]	Prospective case series	Image-guided injection	105	BMC	Yes	Patients failed conservative management
Hernigou et al. [6]	Prospective case controlled	Arthroscopic rotator cuff repair with MSCs *vs* repair only group	45	BMC	Yes	100% healing of tendon on MRI *vs* 67% in control group at 6 months; intact tendon in 87% *vs* 44% at 10 years

BMC bone marrow concentrate, *MSCs* mesenchymal stem cells

Table 40.4 Summary of published research using BMC for hip osteoarthritis

Study	Study type	Intervention	Patients, *n*	Stem cells used	Functional improvement	Notes
Centeno et al. [17]	Prospective case series	Image-guided injection	196	BMC	Yes	Majority of patients were THA candidates
Emadedin et al. [18]	Prospective case series	Unknown	5	Culture-expanded bone marrow MSCs	Yes	Severity unknown

BMC bone marrow concentrate, *MSCs* mesenchymal stem cells, *THA* total hip arthroplasty

40.1.4 Hip Osteoarthritis

The response rates are generally lower in severe disease. Table 40.4 summarizes published research using BMC for hip OA:

- The author's case series of 196 patients treated through injection of BMC demonstrated that patients over age 55 (i.e. likely those with more severe disease) showed poorer outcomes [17].
- Emadedin et al. published a smaller case series of five patients treated with culture-expanded bone marrow MSCs [18].

40.1.5 Lumbar Intervertebral Disc (Degenerative Disc Disease)

Table 40.5 summarizes the published clinical data on BMC for degenerative disc disease (DDD):

- Pettine et al. published 1- and 2-year results, in which patients with the highest MSC dose (as demonstrated by Colony Forming Units) reported the best outcomes [8, 9].
- Other published studies have used isolated and culture-expanded MSCs, autologous nucleus pulposus cells, allogeneic culture-expanded cord blood MSCs, and autologous cord blood MSCs [7, 19, 20].

Table 40.5 Summary of published research using BMC, culture-expanded MSCs, and other cell types to treat degenerative disk disease

Study	Study type	Intervention	Patients, *n*	Stem cells used	Functional improvement	Notes
Mochida et al. [19]	Prospective case series	Surgical implant	9	Autologous nucleus pulposus cells	No—minimal MRI changes	Safety study
Pettine et al. [8, 9]	Prospective case series	Injection into IVD	26	BMC	Yes	Possible slight changes in MRI, but within error of DDD grading scale
Pang et al. [20]	Prospective case series	Surgical implantation	2	Autologous cord blood MSCs	Yes	No imaging
Orozco et al. [7]	Prospective case series	Injection into IVD	10	Autologous culture-expanded bone marrow MSCs	Yes	No improvement in disc height, some increase in T2 signal

BMC bone marrow concentrate, *DDD* degenerative disc disease, *IVD* intervertebral disc, *MSCs* mesenchymal stem cells

40.1.6 Ankle Disorders

- A study published in 2015 that used cultured MSCs for the treatment of osteoarthritis of the ankle showed a significant reduction in pain, improved function, and a decrease in subchondral edema on MRI 6 months after the procedure, in a subset of the patients [18].
- Hernigou et al. [21] published a study on the treatment of ankle non-union in diabetic patients. One arm of the study received the standard treatment with an iliac crest bone graft; the other arm was treated with autologous BMC. Those treated with BMC had a success rate of 82% *versus* 62% in those treated with the bone graft.

40.1.7 Epicondylitis

A case series of 30 patients treated with a single injection of BMC for lateral epicondylitis showed a significant reduction in symptoms at short and medium follow-up intervals [22].

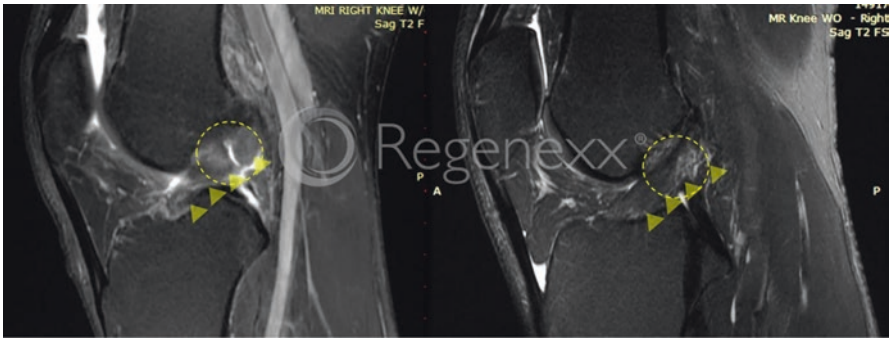

Fig. 40.1 Exemplar MRIs showing improved complete anterior cruciate ligament (ACL) tear signal on sagittal T2 MRI before and 3 months after precise ACL bone marrow concentrate (BMC) injection with fluoroscopic guidance and contrast confirmation. The left image is the pre-procedure knee ACL tear. It shows a white tear (inside the *yellow dashed circle*) diagonally through the dark-appearing ACL. The right image shows the follow-up MRI at 3 months after the injection of the patient's own BMC. No tear is seen inside the *yellow dashed circle*. The *yellow triangles* in both images delineate the course of the ACL

40.1.8 Anterior Cruciate Ligament (ACL) Tears

A small case series published by Centeno et al. [23] demonstrated healing on pre- and post-procedure MRI after treatment with BMC for partial and complete ACL tears (Fig. 40.1).

40.2 Microanatomy and Biochemistry

What is a stem cell? At its most basic, it is a cell that has the following three properties:

- Undifferentiated
- Capable of differentiating into many cell types
- Can divide through mitosis to give rise to new stem cells

The fact that bone marrow contains stem cells was first discovered in the 1960s [24]. Since then, a number of stem cell types that are potentially important to pain management physicians have been discovered:

- *Mesenchymal stem cells (MSCs)*: Multipotent, adult stem cells that show clinical potential as therapeutic agents in regenerative medicine [1–5].
  - Also known as *marrow stromal cells*, these cells are also derived from other mesodermal tissues. MSCs were later assayed and renamed "colony forming fibroblasts" in the 1970s [7].
  - Experiments through the 1980s and 1990s demonstrated that local environmental clues differentiated MSCs into different cell types. For example, cul-

turing these cells with ascorbic acid, inorganic phosphate, or dexamethasone could differentiate cells to osteoblasts, whereas exposure to TGFβ caused cells to differentiate into chondrocytes [1].
  - More recent research has revealed that MSCs are actually a heterogeneous population of similar cells rather than one distinct cell type [8].
  - International groups have attempted to provide a definition of MSC that consists of adherence to plastic, MSC-specific cell surface markers, and multilineage mesodermal tissue differentiation [9].
- *Hematopoietic stem cells (HSCs)*: These cells give rise to the nucleated cells of the blood but also are secondarily involved in muscle repair [25].
  - In the body, HSCs are routinely recruited from the bone marrow when local muscle satellite cells are unable to complete muscle repair.
- *Endothelial progenitor cells (EPCs)*: These cells are recruited from the bone marrow to facilitate vascular homeostasis and neovasculogenesis [26].
  - Because many chronically injured musculoskeletal tissues have poor blood supply, this cell type may be useful for reestablishing vascularity.
- *Pericytes*: These cells are also recruited from the bone marrow for neovasculogenesis, as these cells reside around blood vessels [27].
  - Some believe that pericytes can differentiate into MSCs when injury is detected [28].
- *Osteochondral reticular cells*: These recently discovered cells are concentrated in the metaphysis of long bones, but not in the perisinusoidal space.
  - These cells can differentiate into osteoblasts, chondrocytes, and reticular marrow stromal cells [29].
- *Multilineage-differentiating stress-enduring (MUSE) cells*: These cells can differentiate into all three embryonic layers (endoderm, mesoderm, and ectoderm).
  - These cells act as a reserve cell source; they are difficult to kill and are activated by physical stress. They are also involved in regenerative homeostasis and tissue repair.

40.2.1 Bone Marrow Concentrate Versus Adipose?

Several sources have suggested that adipose contains a higher stem cell count than bone marrow [30, 31]. This misconception seems to be the result of a difference in nucleated cell content between the two tissues:

- Bone marrow has as many or more stem cells per unit volume as adipose tissue.
- Adipose tissue is less cellular and has a much lower nucleated cell content per volume than bone marrow. For example, bone marrow has approximately 100 times more total cells than adipose tissue [32].

- Adipose tissue has a much higher percentage of stem cells, as compared with nucleated cells; 1–5% are MSCs, *versus* 0.01–0.5% in bone marrow.
- Adipose tissue contains significantly fewer HSCs (4×10^{-6}% *versus* 1–2%).

40.3 Basic Concerns and Contraindications

BMC safety in orthopedic diseases has been well established via two large studies:

- Hernigou et al. [33]: 1873 patients monitored for 12.5 years for the occurrence of neoplasm (not all adverse events).
- Centeno et al. [34]: 2372 patients treated at multiple sites, followed for up to 9 years for all adverse events, showing an excellent safety profile. 1835 patients received BMC; the remainder received cultured expanded MSCs.

Common contraindications include anemia, uncontrolled bleeding disorders, and active neoplasm or a history of neoplasm. The paper by Hernigou et al. [33] showed that cancer patients treated with BMC injections for orthopedic conditions did not show any increase in new neoplasm rates. Hence, though injecting MSCs directly into a tumor is ill-advised because of a risk that the cells may differentiate into tumor cells or promote cell proliferation, an existing neoplasm may be a relative contraindication.

40.4 Preoperative Considerations

- Explanation of potential complications and alternative treatments.
- Hematocrit and overall patient health as appropriate for a surgical procedure.
- Anticoagulation and bleeding disorders that may prevent or complicate the clotting process after penetration of the periosteum.
- Physical examination of the area of potential harvest for infection, skin ulceration, or necrosis.
- Heparin produces fewer clots in bone marrow aspirate (BMA) than ACD (acid-citrate-dextrose). It is essential to ensure that the patient has no history of heparin-induced thrombocytopenia. If this is suspected, ACD should be used in place of heparin.

Research on maximizing the MSC yield from BMC has yielded several key points:

- The posterior superior iliac spine contains double the nucleated cells of other bone aspiration sites, like the tibia [35].
- Drawing a large volume (>20 mL) from a single bone site reduces MSC yield; drawing small volumes (5–15 mL) from many sites increases that yield [35].

- MSCs reside in the subcortical areas, and pericytes reside around blood vessels. Hence, drawing from more sites maximizes subcortical MSC yield and allows access to pericytes. Travelling through the bone marrow space sacrifices subcortical MSCs, however.
- Even small concentrations of bupivacaine or lidocaine are toxic to MSCs, so BMA should not be allowed to come in direct contact with either anesthetic. *The only safe drug to use is ropivacaine (0.25% or less)* [36, 37].

40.5 Radiographic Guidance

The hallmark of interventional orthopedics is the use of imaging guidance to precisely place cells into the damaged or diseased tissue. Both ultrasound and fluoroscopic guidance are commonly used. Each has its benefits and its drawbacks.

40.5.1 Ultrasound

- *Benefit:* Superior in imaging superficial soft tissue
- *Drawback:* Unable to image deeper structures obscured by bony tunnels or bone
- *Example*: Suggested for injecting the shoulder rotator cuff, but less than ideal for injecting the knee ACL, because the origin is buried in the trochlear groove.

40.5.2 Fluoroscopy

- *Benefit*: Superior for imaging bone and deeper structures with the aid of contrast
- *Drawback*: Unable to image soft tissues; produces radiation exposure, and cost is higher
- *Example:* Suggested for injecting stem cells into an osteonecrotic lesion of the hip, but would be less appropriate to inject a rotator cuff tear

Attempting bone marrow aspiration without either ultrasound or fluoroscopic guidance is below the standard of care, because the thick areas of the pelvis that have appropriate depth to cannulate are very close to very thin areas that cannot be cannulated (Fig. 40.2).

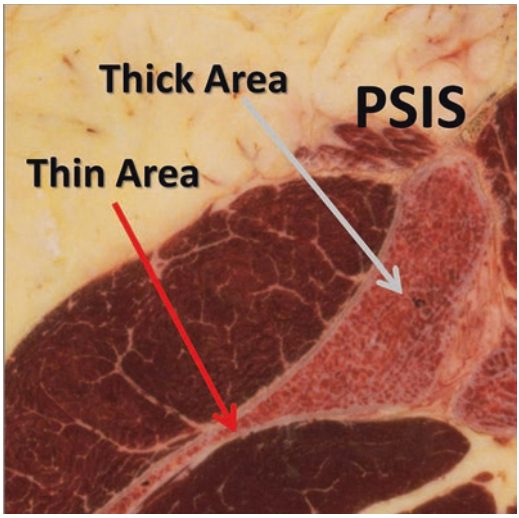

Fig. 40.2 A slice through the bony pelvis showing two marrow draw angles. The first goes through the "thin area" or the area identified as more radiolucent on plain radiographs. This is a thin area of the pelvis, where the likelihood of passing through the marrow space is very high. The "thick area" is a more opaque area on radiographs. This area has a large marrow space with less risk of passing through the marrow-rich area and much higher likelihood of aspirating whole marrow. *PSIS* posterior superior iliac spine

40.6 Equipment

- 10–15 mL of 1% lidocaine or 0.25% ropivacaine
- 5000 IU vial of heparin
- 20,000 IU and 10,000 IU vials of heparin
- Preservative-free normal saline
- 30 G needle (for anesthetizing skin)
- 25 G 3.5-in. spinal needle (for anesthetizing periosteum and underlying tissue)
- Sterile 11-G disposable trocars (one for each side of access)
- 5-mL syringe
- 30-mL syringes
  - At least one study has suggested that using multiple 5- or 10-mL syringes may increase MSC yield [38]. We have been unable to replicate these results, which may be an artifact of the larger 50-mL syringes used as comparison.

Most physicians using BMC utilize a commercially available 510 K approved bedside centrifuge, which uses a disposable kit, such as those systems listed here:

- Accelerate: Autologous Platelet Concentrating System
- Accellerated Biologics: BC 60 and BC 120 Pure
- Arthrex Angel

- Celling ART BMC
- EmCyte 544E
- EmCyte PureBMC
- GenesisCS Component Concentrating System
- Harvest Technologies SmartPReP 2
- ISTO CellPoint

Some physicians also use manual processing and a biologic safety cabinet. The advantages of a bedside centrifuge using a kit are ease of use, lower start-up costs, and a requirement for very little training. The disadvantage is that frequently a standard volume is inputted and a standard volume is delivered in this one-size-fits-all approach.

40.7 Technique

40.7.1 Harvesting BMC

Bone marrow can be obtained easily via an aspirate procedure. This is a safe procedure; one large registry in the UK, which included bone marrow aspiration and trephine biopsy, showed 15 serious adverse events in 20,323 procedures [39].

The suggested target for harvesting BMC is the pelvic crest. The steps for bone marrow aspiration are as follows:

- The patient is placed prone on a procedure table.
- After sterile prep, 10–15 mL of either 1% lidocaine (which must not come into contact with the BMA) or 0.25% ropivacaine are injected.
  - It is critical for the numbing to occur under guidance, with careful attention paid to the exact areas that are injected; straying out of the anesthetized area will cause significant pain. It is also critical that the skin, soft tissues, and periosteum are injected. In my experience with more than 1000 bone marrow aspiration procedures, the single biggest cause of pain is inadequate numbing of the soft tissues.
- Sufficient time must be allowed to pass for the area to be adequately anesthetized.
- Prep the draw syringes by adding 1000 units of heparin per milliliter drawn (or follow the instructions of the point-of-care automated centrifuge). Thus 30-mL syringes would have 30,000 units of heparin per syringe.
- Prep one additional 5-mL syringe with 5000 units of heparin in normal saline.
- If using ultrasound, the entry must be at a shallow angle to the probe (Fig. 40.3). If using fluoroscopy, the entry point is at a steeper angle (Fig. 40.4). The draw sites are around the posterior superior iliac spine (PSIS), as shown in Fig. 40.5. These target the thick areas of the bone marrow and avoid the thin areas.

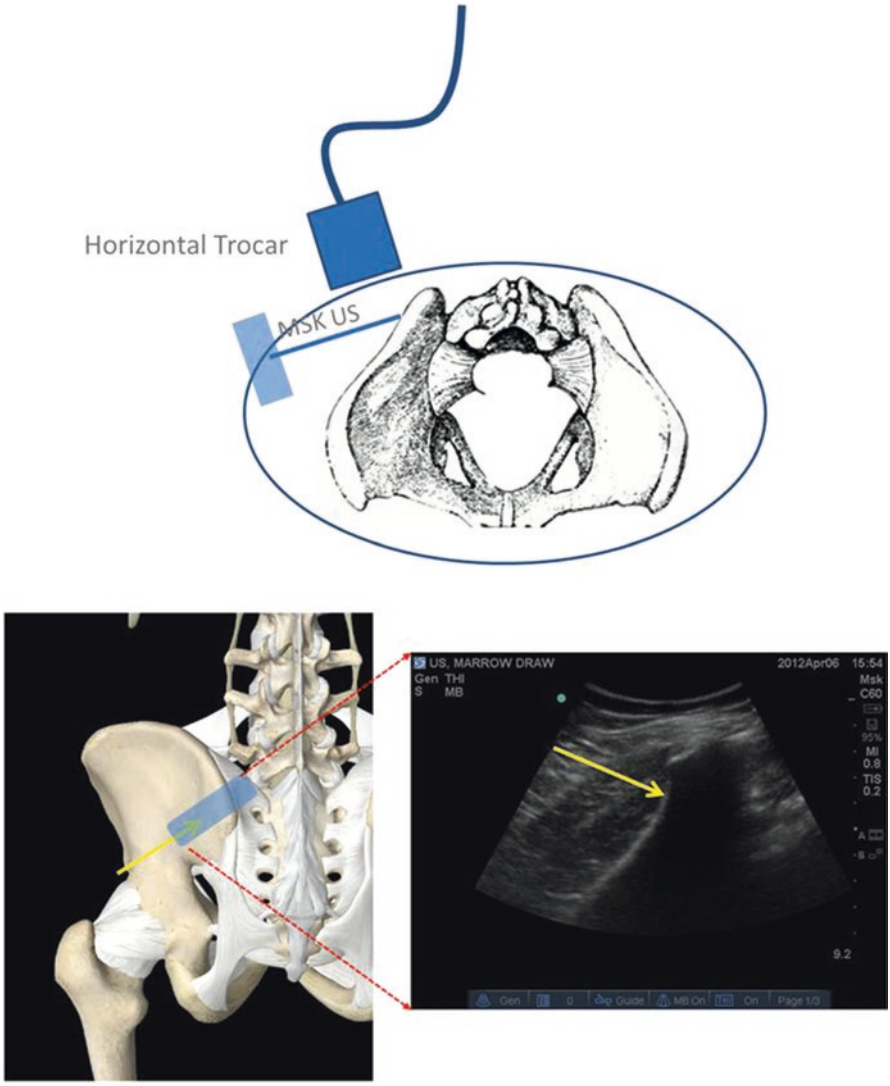

Fig. 40.3 The ideal ultrasound linear probe and trocar placement for identification of the posterior superior iliac spine (PSIS) during a bone marrow aspiration procedure. *MSK US* musculoskeletal ultrasound

- The trocar is placed against the bone cortex, and forward pressure is used while the device is turned back and forth, using the angled tip to bore a hole in the bone. The trocar is advanced 5–10 mm until it is seated in the cortex. Note that many trocars have 1-cm markers on the shaft, making it easy to gauge the depth.

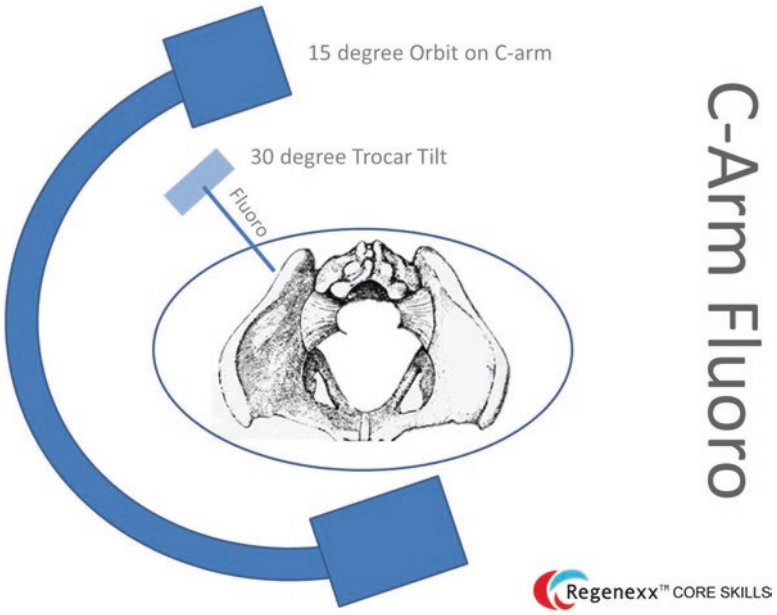

Fig. 40.4 The ideal fluoroscopic C-arm and trocar placement for identification of the PSIS during a bone marrow aspiration procedure

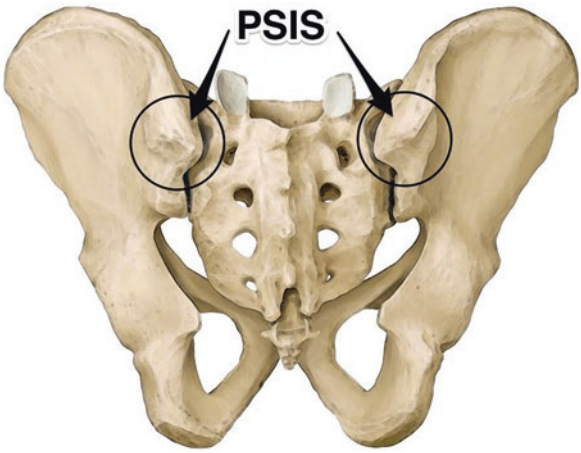

Fig. 40.5 Depiction of the PSIS located on the posterior pelvis. This is the ideal area for three or four draw sites from each PSIS

- To ensure that the trocar is properly seated in the bone, the trocar should be wiggled gently back and forth; if solid, no further advancement is required. If it can still be moved, then the trocar is advanced until it is solidly in the bone.
- Remove the stylet from the trocar and make sure the trocar is still firmly implanted in the cortex by performing a second wiggle test. If it is not firmly implanted, advance the trocar until firm, not exceeding approximately 1 cm in depth.

- Because clotting of the marrow sample traps unrecoverable MSCs in the clot, avoiding this event is important. Hence, before aspiration, the 5-mL syringe with the 5000 units of heparin is placed on the trocar and approximately 500–750 units of heparin is injected into the marrow space immediately on entry into the cortex. This is performed for each bone site entered.
- Attach the draw syringe to the trocar. Pull back on the plunger to patient tolerance. The BMA will not naturally mix with the heparin, so the first few milliliters of BMA must be mixed with the heparin using gentle agitation of the syringe.
- Once mixed, draw no more than 5–15 mL per site. Once complete, move the trocar tip to a new cortex site, using the same skin site (i.e., not removing the trocar from the skin).
- Draw volumes are dependent on patient weight and the size of the area to be treated.
  - For women less than 105 pounds, draw no more than 50 mL.
  - For women between 105 and 120 pounds, the most appropriate draw volume is 60 mL.
  - For heavier patients of either sex between 120 and 180 pounds, up to 90 mL can be drawn.
  - For men over 180 pounds, 120 mL can be drawn.

40.7.2 Processing BMC

There are many commercial systems to process BMA. Some physicians also process via manual means in a biologic safety cabinet. The goal of BMA concentration is to isolate the buffy coat, which is the smaller, grey, middle section in a centrifuged BMA sample. Automated bedside systems all concentrate the buffy coat and have developed many proprietary ways to perform this simple task. To date, little third-party research is available comparing the MSC outputs of various concentration devices.

40.7.3 Dosing BMC for Use

The dose of BMC can be quantified as follows:

- *Colony Forming Unit (CFU) assay*: BMC is placed in monolayer culture and incubated until colonies of plastic-adherent MSCs form. These are then counted as a rough metric of MSC content [40].
  - This technique is helpful for research, but is little help to the clinician at the bedside.
- *Flow cytometry*: The cells in BMC are stained via fluorescent antibodies to specific cell surface markers and run through a flow cytometer, which uses laser light

to identify collections of markers. MSCs have a known marker panel, commonly considered to be CD34−, CD14−, CD105+, CD44+, CD90+, CD73+ [41].

  - This technique can be used clinically, but the expertise required to run and analyze the results and the cost of this technology make it unlikely to be used for most clinical settings.

- *Total Nucleated Cell Count (TNC)*: The number of nucleated cells in the BMC is counted using either a manual hemocytometer or a commercial automated counting system [42].

  - TNC can be used at the bedside for clinical settings. Note that it is not a direct MSC count, but an indirect measure of a proxy for that count.

The research on dosing of BMC and clinical outcome has consistently shown that higher CFU counts or TNC counts are associated with better clinical outcome [9].

40.8 Postoperative Considerations

Our extensive experience in culturing MSCs using an autologous platelet lysate procedure has taught us that certain medications can cause cell culture failure and hence reduce MSC function. These medications should be stopped for two to three serum half-lives before a BMC procedure and at least 2–4 weeks after the procedure, where feasible:

- Nonsteroidal anti-inflammatory drugs (NSAIDs) [43]
- Corticosteroids [44]
- ACE inhibitors [45]
- Statins [46]

40.9 Potential Complications and Pitfalls

- Marcaine, bupivacaine, and lidocaine are toxic to MSCs at low concentrations. Injecting these anesthetics with BMC will significantly reduce cell viability. Ropivacaine at concentrations of 0.125–0.25% is safe to use with MSCs [36, 37].
- Incomplete anesthesia of the periosteum can lead to intense pain and even neuralgia.
  - Many clinicians numb only the skin and then the deep tissues. It is critical also to numb the mid-field soft tissues between these two areas.
  - The clinician MUST provide adequate time for the local anesthetic to take effect, typically 3–5 min. Numbing these tissues first on one side and then the other, and then drawing medications, usually provides that set time.

- When using fluoroscopy, there is an innate sense of two-dimensional anatomy, so simply remembering anatomic landmarks will help define that area.
- When using ultrasound, the cross-sectional nature of the imaging technology provides less information about the location of anesthetic. We suggest using a sterile surgical marker on the skin to better define the numbed area.
- Many physicians have been taught to pull high volumes (60 mL or more) from a single site during bone marrow aspiration. Doing so will dramatically reduce yield, as discussed above.
- Most are unaware that clots can form in the BMA and will reduce MSC yield. These clots can form when not using heparin in the BMA or not pre-heparinizing the draw sites. We suggest using heparin because it is a much more effective anticoagulant than ACD. It must be used in the BMA draw syringe (see above) and must be mixed as soon as the BMA hits the syringe, as it will not naturally mix through diffusion. In addition, the immediate injection of heparin directly into the bone site being cannulated, before the BMA is drawn, will help prevent clots in slow bone marrow aspirations due to dehydration.

40.10 Clinical Pearls

- Adipose tissue does not necessarily yield higher counts of MSCs.
- Anesthetics take time to work. The patient can be made more comfortable simply by injecting local anesthetic first, then drawing up heparin and preparing the trocar, then placing the trocar into the skin. These extra few minutes of prep time allow for a better block. Avoid using lidocaine or bupivacaine, which can be toxic to the MSCs; instead use ropivacaine or prevent the anesthetic from coming into contact with the graft.
- Using multiple collection syringes for several smaller aspirations rather than one larger one will allow for higher yields.
- Heparin is preferred over ACD because it prevents clotting, which may potentially decrease cell counts.

References

1. Centeno CJ, Al-Sayegh H, Bashir J, Goodyear S, Freeman MD. A prospective multi-site registry study of a specific protocol of autologous bone marrow concentrate for the treatment of shoulder rotator cuff tears and osteoarthritis. J Pain Res. 2015;8:269–76.
2. Daltro GC, Fortuna V, de Souza ES, Salles MM, Carreira AC, Meyer R, et al. Efficacy of autologous stem cell-based therapy for osteonecrosis of the femoral head in sickle cell disease: a five-year follow-up study. Stem Cell Res Ther. 2015;6:110.
3. Gobbi A, Scotti C, Karnatzikos G, Mudhigere A, Castro M, Peretti GM. One-step surgery with multipotent stem cells and Hyaluronan-based scaffold for the treatment of full-thickness chondral defects of the knee in patients older than 45 years. Knee Surg Sports Traumatol Arthrosc. 2016;25(8):2494–501.

4. Gobbi A, Whyte GP. One-stage cartilage repair using a hyaluronic acid-based scaffold with activated bone marrow-derived mesenchymal stem cells compared with microfracture: five-year follow-up. Am J Sports Med. 2016;44:2846–54.
5. Havlas V, Kotaška J, Koníček P, Trč T, Konrádová Š, Kočí Z, Syková E. Use of cultured human autologous bone marrow stem cells in repair of a rotator cuff tear: preliminary results of a safety study. Acta Chir Orthop Traumatol Cechoslov. 2015;82:229–34.
6. Hernigou P, Flouzat Lachaniette CH, Delambre J, Zilber S, Duffiet P, Chevallier N, Rouard H. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: a case-controlled study. Int Orthop. 2014;38:1811–8.
7. Orozco L, Soler R, Morera C, Alberca M, Sánchez A, García-Sancho J. Intervertebral disc repair by autologous mesenchymal bone marrow cells: a pilot study. Transplantation. 2011;92:822–8.
8. Pettine K, Suzuki R, Sand T, Murphy M. Treatment of discogenic back pain with autologous bone marrow concentrate injection with minimum two year follow-up. Int Orthop. 2016;40:135–40.
9. 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;33:146–56.
10. Zhao D, Liu B, Wang B, Yang L, Xie H, Huang S, et al. Autologous bone marrow mesenchymal stem cells associated with tantalum rod implantation and vascularized iliac grafting for the treatment of end-stage osteonecrosis of the femoral head. Biomed Res Int. 2015;2015:240506.
11. Sampson S, Botto-van Bemden A, Aufiero D. Autologous bone marrow concentrate: review and application of a novel intra-articular orthobiologic for cartilage disease. Phys Sportsmed. 2013;41:7–18.
12. Hernigou P, Poignard A, Zilber S, Rouard H. Cell therapy of hip osteonecrosis with autologous bone marrow grafting. Indian J Orthop. 2009;43:40–5.
13. Vangsness CT Jr, Farr J II, Boyd J, Dellaero DT, Mills CR, LeRoux-Williams M. Adult human mesenchymal stem cells delivered via intra-articular injection to the knee following partial medial meniscectomy: a randomized, double-blind, controlled study. J Bone Joint Surg Am. 2014;96:90–8.
14. Vega A, Martín-Ferrero MA, Del Canto F, Alberca M, García V, Munar A, et al. Treatment of knee osteoarthritis with allogeneic bone marrow mesenchymal stem cells: a randomized controlled trial. Transplantation. 2015;99:1681–90.
15. Centeno C, Pitts J, Al-Sayegh H, Freeman M. Efficacy of autologous bone marrow concentrate for knee osteoarthritis with and without adipose graft. Biomed Res Int 2014;2014:370621.
16. Kim YS, Choi YJ, Koh YG. Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical outcomes. Am J Sports Med. 2015;43:2293–301.
17. Centeno CJ, Pitts JA, Al-Sayegh H, Freeman MD. Efficacy and safety of bone marrow concentrate for osteoarthritis of the hip; treatment registry results for 196 patients. J Stem Cell Res Ther. 2014;4:242.
18. Emadedin M, Ghorbani Liastani M, Fazeli R, Mohseni F, Moghadasali R, et al. Long-term follow-up of intra-articular injection of autologous mesenchymal stem cells in patients with knee, ankle, or hip osteoarthritis. Arch Iran Med. 2015;18:336–44.
19. Mochida J, Sakai D, Nakamura Y, Watanabe T, Yamamoto Y, Kato S. Intervertebral disc repair with activated nucleus pulposus cell transplantation: a three-year, prospective clinical study of its safety. Eur Cell Mater. 2015;29:202–12; discussion 212.
20. Pang X, Yang H, Peng B. Human umbilical cord mesenchymal stem cell transplantation for the treatment of chronic discogenic low back pain. Pain Physician. 2014;17:E525–30.
21. Hernigou P, Guissou I, Homma Y, Poignard A, Chevallier N, Rouard H, Flouzat Lachaniette CH. Percutaneous injection of bone marrow mesenchymal stem cells for ankle non-unions decreases complications in patients with diabetes. Int Orthop. 2015;39:1639–43.
22. Singh A, Gangwar DS, Singh S. Bone marrow injection: a novel treatment for tennis elbow. J Nat Sci Biol Med. 2014;5:389–91.

23. Centeno CJ, Pitts J, Al-Sayegh H, Freeman MD. Anterior cruciate ligament tears treated with percutaneous injection of autologous bone marrow nucleated cells: a case series. J Pain Res. 2015;8:437–47.
24. Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3:393–403.
25. Strömberg A, Jansson M, Fischer H, Rullman E, Hägglund H, Gustafsson T. Bone marrow derived cells in adult skeletal muscle tissue in humans. Skelet Muscle. 2013;3:12.
26. Szmitko PE, Wang CH, Weisel RD, Jeffries GA, Anderson TJ, Verma S. Biomarkers of vascular disease linking inflammation to endothelial activation: part II. Circulation. 2003;108:2041–8.
27. Bergmann CE, Hoefer IE, Meder B, Roth H, van Royen N, Breit SM, et al. Arteriogenesis depends on circulating monocytes and macrophage accumulation and is severely depressed in op/op mice. J Leukoc Biol. 2006;80:59–65.
28. Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;3:301–13.
29. Worthley DL, Churchill M, Compton JT, Tailor Y, Rao M, Si Y, et al. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. Cell. 2015;160:269–84.
30. Mafi R, Hindocha S, Mafi P, Griffin M, Khan WS. Sources of adult mesenchymal stem cells applicable for musculoskeletal applications - a systematic review of the literature. Open Orthop J. 2011;5(Suppl 2):242–8.
31. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7:211–28.
32. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum. 2005;52:2521–9.
33. Hernigou P, Homma Y, Flouzat-Lachaniette CH, Poignard A, Chevallier N, Rouard H. Cancer risk is not increased in patients treated for orthopaedic diseases with autologous bone marrow cell concentrate. J Bone Joint Surg Am. 2013;95:2215–21.
34. Centeno CJ, Al-Sayegh H, Freeman MD, Smith J, Murrell WD, Bubnov R. A multi-center analysis of adverse events among two thousand, three hundred and seventy two adult patients undergoing adult autologous stem cell therapy for orthopaedic conditions. Int Orthop. 2016;40:1755–65.
35. Marx RE, Tursun R. A qualitative and quantitative analysis of autologous human multipotent adult stem cells derived from three anatomic areas by marrow aspiration: tibia, anterior ilium, and posterior ilium. Int J Oral Maxillofac Implants. 2013;28:e290–4.
36. Breu A, Eckl S, Zink W, Kujat R, Angele P. Cytotoxicity of local anesthetics on human mesenchymal stem cells in vitro. Arthroscopy. 2013;29:1676–84.
37. Dregalla RC, Lyons NF, Reischling PD, Centeno CJ. Amide-type local anesthetics and human mesenchymal stem cells: clinical implications for stem cell therapy. Stem Cells Transl Med. 2014;3:365–74.
38. Hernigou P, Homma Y, Flouzat Lachaniette CH, Poignard A, Allain J, Chevallier N, Rouard H. Benefits of small volume and small syringe for bone marrow aspirations of mesenchymal stem cells. Int Orthop. 2013;37:2279–87.
39. Bain BJ. Morbidity associated with bone marrow aspiration and trephine biopsy – a review of UK data for 2004. Haematologica. 2006;91:1293–4.
40. Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay ofcells in vitro. Nat Protoc. 2006;1:2315–9.
41. Boxall SA, Jones E. Markers for characterization of bone marrow multipotential stromal cells. Stem Cells Int. 2012;2012:975871.
42. Peters AE, Watts AE. Biopsy needle advancement during bone marrow aspiration increases mesenchymal stem cell concentration. Front Vet Sci. 2016;3:23.
43. Chang JK, Li CJ, Wu SC, Yeh CH, Chen CH, Fu YC, et al. Effects of anti-inflammatory drugs on proliferation, cytotoxicity and osteogenesis in bone marrow mesenchymal stem cells. Biochem Pharmacol. 2007;74:1371–82.

44. Wyles CC, Houdek MT, Wyles SP, Wagner ER, Behfar A, Sierra RJ. Differential cytotoxicity of corticosteroids on human mesenchymal stem cells. Clin Orthop Relat Res. 2015;473:1155–64.
45. Durik M, Sevá Pessôa B, Roks AJ. The renin-angiotensin system, bone marrow and progenitor cells. Clin Sci (Lond). 2012;123:205–23.
46. Izadpanah R, Schächtele DJ, Pfnür AB, Lin D, Slakey DP, Kadowitz PJ, Alt EU. The impact of statins on biological characteristics of stem cells provides a novel explanation for their pleiotropic beneficial and adverse clinical effects. Am J Physiol Cell Physiol. 2015;309:C522–31.