Bone Marrow-Derived Stem Cells and Their Application in Pain Medicine

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Introduction

In recent years, there has been growing interest in the use of bone marrow in the treatment of various musculoskeletal disorders based on its possible regenerative capabilities. The most common type of therapy uses bone marrow concentrate (BMC) obtained by isolating the buffy coat found within centrifuged bone marrow aspirate [1]. Bone marrow is a good source of mesenchymal stem cells (MSCs), which play a vital role in repair process for damaged musculoskeletal tissues [2]. MSCs have been shown to play a role in tissue healing through their ability to mobilize to the site of damaged tissue and differentiate into other mesenchymal precursors, as well as signal neighboring cells to assist in repair. Early clinical data show the clinical use of MSCs in the treatment of knee, hip, and shoulder osteoarthritis as well as intervertebral disc disease [3–12].

Incorporating BMC into clinical treatment options has the potential to create a shift in the treatment of musculoskeletal injuries, from traditional orthopedic surgery focused on removing or modifying tissue to precise, image-guided injections to facilitate healing of injured or damaged soft tissue and bone. The potential advantages of using a regenerative approach to treat musculoskeletal conditions include decreased procedural risk when compared with surgical alternatives, lessened post-procedural morbidity, and decreased healthcare cost. This approach has many implications for pain management clinicians as their interventional skill sets allow for the precise administration of BMC preparations into a specific structure of need.

Microanatomy and Biochemistry

The following three properties help describe stem cells:

- Undifferentiated
- Capable of cell differentiation
- Capable of cell division through mitosis.

Bone marrow was first discovered to be a source of mesenchymal stem cells in the 1960s [13]. Since then, there have been many advances in our understanding of the MSC's role in tissue repair. In addition, several other bone marrow cell types have been studied, all of which may have significant clinical implications in the future.

- *Mesenchymal stem cells (MSCs):* adult stem cells which are multipotent, capable of dividing into progeny that give rise to all skeletal tissue types including cartilage, bone, tendon, ligament, and connective tissue [14].
  - MSCs are derived from other mesodermal tissues and are also known as marrow stromal cells and later assayed and renamed "colony-forming fibroblasts" in the 1970s [14].
  - Numbered by colony-forming units (CFUs)
  - MSCs are a heterogeneous population of similar cells rather than one distinct cell type.

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– Several international groups have provided criteria for identifying MSCs in the research and clinical setting. A mesenchymal stem cell must demonstrate [15]
  • Adherence to plastic
  • Cell surface markers specific to MSCs
  • Multi-lineage mesodermal tissue differentiation.
– There are several unique properties of MSCs which provide a physiologic basis for their clinical application in regenerative medicine for orthopedic applications.
  • MSCs respond to local environmental stimuli, signaling them to differentiate into their various terminal cell types (for example, culturing these cells with ascorbic acid, inorganic phosphate, or dexamethasone could differentiate cells to osteoblasts, while exposure to TGF-beta caused cells to differentiate into chondrocytes) [16].
  • MSCs also participate in paracrine signaling prompting neighboring cells to participate in tissue repair [2, 17].
  • They have also shown to be capable of mobilizing through the peripheral circulation to distant sites of injury in a mouse model [18]

• *Hematopoietic stem cells (HSCs)*: primarily give rise to nucleated cells of the blood and may be secondarily involved in muscle repair [19].
  – Satellite cells recruit HSCs to the local area from the bone marrow reservoir when muscle repair is incomplete.
• *Endothelial progenitor cells (EPCs):* recruited from bone marrow to facilitate vascular homeostasis and neovasculogenesis [20].
  – Musculoskeletal tissue that has suffered chronic injury and is unable to completely heal may have poor blood supply. EPCs may aid in re-establishing vascularity through secreting vascular endothelial growth factor (VEGF).
• *Pericytes*: located near blood vessels and recruited from bone marrow to promote neovasculogenesis and tissue repair [21].
  – Research suggests pericytes may differentiate into MSCs when injury is detected [22].
• *Osteochondral reticular cells (ORCs)*: recently discovered and concentrated in the metaphysis of long bones. Hence, these are not found in BMA, but may be found in other bone marrow procedures that involve bone grafts.
  – ORCs differentiate into osteoblasts, chondrocytes, and reticular marrow stromal cells [23].
• *Multilineage Differentiating Stress Enduring (MUSE) Cells:* capable of differentiating between all three embryonic layers (endoderm, mesoderm, and ectoderm).
  – Activated by physical stress, MUSE cells act as a progenitor reserve cell source, in part because they survive longer and harsher environments than many other cell types. They are also involved in regenerative homeostasis and tissue repair.

BMC vs. Adipose

Controversy exists as to which tissue type provides the best source of mesenchymal stem cells. Several studies suggest that adipose tissue contains a higher stem cell count when compared to bone marrow [24, 25]. However, this is largely a misconception due to difference in interpretation of cell content between the two tissues.

• Adipose tissue has a higher percentage of MSCs as compared to nucleated cells.
  – Adipose tissue: 1–5% of nucleated cells are MSCs.
  – Bone marrow: 0.01–0.5% of nucleated cells are MSCs.
• However, bone marrow has approximately 100 times more total nucleated cells (TNCs) than adipose tissue [26] per volume.
• Also, adipose tissue contains significantly fewer HSCs, which give rise to nucleated blood cells and play a role in muscle repair as mentioned above.
  – Generally, bone marrow contains the same or more total stem cells per unit volume compared to adipose tissue.

Indications

As of August 2019, the total number of patients treated with bone marrow stem cells for orthopedic conditions that have been published in the U.S. Library of Medicine therapy was 11,467. The number was obtained by summing the *n* of all clinical studies that used either bone marrow concentrate or culture expanded MSCs.

The following indications represent the majority of clinical outcome data available using BMC:

• Osteonecrosis
  – Hernigou et al. published the largest study to date (*n* = 342) using core decompression + autologous BMC in treatment of osteonecrosis of the hip [27]
    • ARCO grade 1–2: showed approximately an 80% long-term likelihood of not requiring hip arthroplasty.
    • ARCO grade 3–4: there was declining success.
• Knee Osteoarthritis:
  – Vagness et al. reported approximately one in four patients demonstrated an increase in meniscus size [28].
  – Vega found significant improvement in cartilage signal on follow-up T2 MRI sequences [29]

- Centeno et al. published a large case series demonstrating improved pain/functional outcomes regarding knee OA. Also, it was found that the addition of a fat graft does not improve outcomes over injecting BMC alone [30].
- Hernigou has published two works focused on intraosseous injection of BMC [31, 32]. In one randomized trial, he injected knee osteoarthritis patients on one side with intra-articular BMC injection versus the other side with intraosseous BMC injection (IO). The IO injection had fewer patients convert to knee arthroplasty at 15 years. In a second trial, he compared the efficacy of IO BMC injection to knee arthroplasty at 15 years and found good results for the majority of those treated with BMC. Those patients with more bone marrow edema fared more poorly on long-term follow-up (Table 4.1).

• Hip Osteoarthritis (Table 4.2):

- Based on the author's experience and unpublished registry data, severe disease yields lower response rates, on average.
- Centeno et al. reported on a case series of 196 patients treated with BMC injection. Poorer outcomes were found for patients over 55. It is suspected that these patients likely had more severe underlining disease [42].
- Emadedin et al. performed a small case series of five patients treated with culture expanded bone marrow MSCs and reported functional improvement [40].
- Rivera has published a prospective comparison of surgical BMC use to treat hip femoroacetabular impingement to a retrospective cohort of surgically treated patients. The author found more efficacy for the BMC-treated group [41].

• Shoulder Rotator Cuff (Table 4.3):

Table 4.1 Summary of selected published research using bone marrow concentrate or culture expanded bone marrow mesenchymal stem cells for knee osteoarthritis

| Author	Study type	Intervention	Patient *n*	Stem cells used	Functional improvement	Notes
Vangsness [28]	Randomized controlled trial	Partial meniscectomy with MSC injection vs. placebo	55	Allogeneic cultured bone marrow MSCs	Yes	1 in 4 patients with increased meniscus volume
Centeno [33]	Randomized controlled trial	Image-guided injection vs. physical therapy	48	Autologous bone marrow concentrate	Yes	Cross over with physical therapy at 3 months
Centeno [30]	Prospective case series	Image-guided injection	840	Autologous bone marrow concentrate	Yes	⅔ of patients were knee arthroplasty+ candidates
Kim [34]	Prospective case series	Injection	49	Autologous cultured bone marrow MSCs	Yes	Full-thickness chondral lesions <6 cm responded best
Vega [29]	Randomized controlled trial	Injection of MSCs vs. hyaluronic acid	30	Allogeneic cultured bone marrow MSCs	Yes	Improved cartilage signal on MRI T2 mapping
Teo [35]	Prospective case series	Surgical implantation of MSCs vs. first generation autologous chondrocyte implantation	36	Autologous cultured bone marrow MSCs	Yes	MSCs equivalent to autologous chondrocyte implantation
Mautner [36]	Case series	Injection of BMC vs. microfragmented adipose tissue (Mfat)	41	Autologous bone marrow concentrate	Yes	No difference between BMC and Mfat
Gobbi [37]	Case series	Surgical implantation of bone marrow concentrate plus hyaluronic acid	23	Autologous bone marrow concentrate	Yes	Results not dependent on chondral lesion size or location
Kim [38]	Case series	High tibial osteotomy with surgical implantation of MSCs alone vs. MSCs+ allogeneic cartilage implant	80	Allogeneic cultured bone marrow MSCs	Yes	MSCs+cartilage better than MSCs alone
Hernigou [31]	Randomized controlled trial	One knee injected intra-articular and the other injected intraosseous	60	Bone marrow concentrate	Yes	Intraosseous alone superior for preventing the need for knee arthroplasty at 15 years
Hernigou [32]	Randomized controlled trial	One knee had knee arthoplasty and the other intraosseous injection	140	Bone marrow concentrate	Yes	Intraosseous alone helped most patients avoid the need for TKA on the non-operated side

MSCs mesenchymal stem cells

Table 4.2 Summary of published research using bone marrow concentrate or cultured expanded bone marrow mesenchymal stem cells for hip osteoarthritis

Author	Study type	Intervention	Patient n	Stem cells used	Functional improvement	Notes
Centeno [39]	Prospective case series	Image-guided injection	196	Bone marrow concentrate	Yes	Majority of patients were hip arthroplasty candidates
Emadedin [40]	Prospective case series	Unknown	5	Culture expanded	Yes	Severity of hip osteoarthritis unknown
Rivera [41]	Prospective case series compared to retrospective cohort	Arthroscopy with or without bone marrow concentrate	80	Bone marrow concentrate	Yes	Adding bone marrow concentrate helped surgical outcomes

Table 4.3 Summary of published research using bone marrow concentrate for shoulder osteoarthritis and rotator cuff tear

Author	Study type	Intervention	Patient n	Stem cells used	Functional improvement	Notes
Centeno [3]	Prospective case series	Image-guided injection	105	Bone marrow concentrate	Yes	Patients failed conservative management
Centeno [43]	Randomized controlled trial	Image-guided injection	25	Bone marrow concentrate	Yes	Bone marrow concentrate better than physical therapy
Hernigou [8]	Prospective case controlled	Arthroscopic rotator cuff repair with bone marrow concentrate vs. surgical repair only	45	Bone marrow concentrate	Yes	100% healing of tendon on MRI vs. 67% in control group at 6 months and intact tendon in 87% vs. 44% at 10 years

– In a comparison trial of surgical repair with and without injected BMC, patients injected with BMC experienced a re-tear rate 50% less than the surgery only group [8].

– Centeno et al. demonstrated significant reductions in pain and increases in validated functional metrics through a case series of 102 patients who had both shoulder OA and a rotator cuff tear [3] In a follow-on RCT, the same author demonstrated good results when using bone marrow concentrate to treat rotator cuff tears in a cross-over RCT with physical therapy alone as a comparator (see Fig. 4.1) [43].

• Lumbar Intervertebral Disc—Degenerative Disc Disease (DDD) (Table 4.4):

– Pettine et al. published showed that higher MSC (CFUs) doses corresponded to the best outcomes at 1- and 2-year results [10, 11].

– Orozco et al. treated ten patients with chronic low back pain and disc degeneration with culture-expanded MSCs from BMC and found statistically significant improvements in pain and function which were sustained at 1 year [9].

– In another study, nine patients were injected with autologous BM-MSCs that were co-cultured with nucleus pulposus cells, into Pfirrmann grade III degenerated discs adjacent to spinal fusion levels. It showed that there was no progression of disc degeneration in adjacent segments to spinal fusion over a 3 -year follow-up time period [9, 44, 46].

– Finally, Noriega injected degenerative discs with allogeneic MSCs and found that a responder cohort of about 40% reported significant decreases in pain and improve-
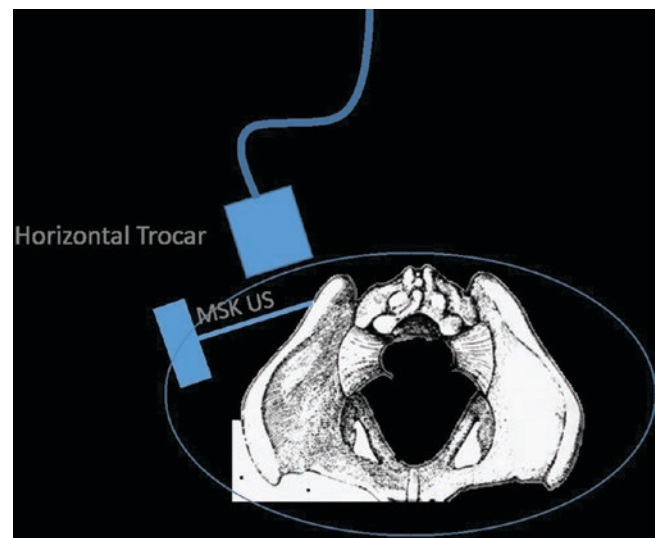
Fig. 4.1 Illustration of ideal ultrasound linear probe and trocar placement for identification of the posterior superior iliac spine during a bone marrow aspiration procedure

ments in function [49]. This concept of a "responder cohort" for DDD patients treated with MSCs is also consistent with non-peer-reviewed data presented via press release by Mesoblast, a company pursuing FDA approval for allogeneic bone marrow MSCs.

• Ankle Disorders:

– Emadedin et al. treated ankle osteoarthritis with cultured MSCs and reported a significant reduction in pain as well as subchondral edema on MRI 6 months post-procedure. In addition, there was improved function [40].

Table 4.4 A summary of BMC and culture expanded mesenchymal stem cells and other cell type-treated degenerative disc disease patients

Author	Study type	Intervention	Patient n	Stem cells used	Functional improvement	Notes
Mochida [44]	Prospective case series	Surgical implant	9	Autologous nucleus pulposus cells	No-minimal MRI changes	Safety study
Pettine [45]	Prospective case series	Image-guided intradiscal injection	26	Bone marrow concentrate	Yes	Possible slight changes in MRI, but within error of degenerative disc disease grading scale
Pang [46]	Prospective case series	Surgical implantation	2	Allogeneic cord blood MSCs	Yes	No imaging
Orozco [9]	Prospective case series	Image-guided intradiscal injection	10	Autologous culture expanded bone marrow-MSCs	Yes	No improvement in disc height, some decrease in T2 signal
Centeno [47]	Prospective Case series	Image-guided intradiscal injection	33	Autologous culture expanded bone marrow-MSCs	Yes	Improvements in disc bulge size
Elabd, Centeno [48]	Prospective Case series	Image-guided intradiscal injection	5	Autologous culture expanded bone marrow-MSCs	Yes	Improvements in disc bulge size
Noriega [49]	Randomized controlled trial	Image-guided intradiscal injection vs. sham injection into paravertebral muscles	24	Allogeneic culture expanded bone marrow MSCs	Yes	Only a group of "responders" at 40% of the treatment cohort had positive results

MSCs mesenchymal stem cells

- – Hernigou et al. published a large study comparing 86 diabetic ankle fracture non-union patients treated with BM-MSCs vs. 86 treated traditionally with iliac bone graft. Patients receiving traditional treatment with iliac crest bone graft had a 62% healing rate, whereas those treated with BM-MSCs had a success rate of 82% and fewer complications [50].
- Epicondylitis:
  - – Singh et al. performed a case series of 30 patients treated with a single injection of BMC for lateral epicondylitis. The report showed a significant reduction in symptoms at short and medium follow-up intervals [51].

Safety Profile and Contraindications

Two large studies have demonstrated the safety of orthopedic conditions treated with BMC.

- In 2013, Hernigou et al. published findings on 1873 patients that had been monitored for an average of 12.5 years and found incidence of neoplasm in the area of BMC injection [52].
- In 2016, Centeno et al. published findings for 2372 patients who had been treated at multiple clinic sites with either BMC or culture expanded MSCs and followed for up to 9 years regarding all adverse events. They reported a 1.5% incidence of serious adverse events and a lower incidence of neoplasm over the course of follow-up than that occurs in the general population [53].

Contraindications include

- Anemia
- Coagulopathy
- Active or history of neoplasm (Relative contraindications)
  - – Cancer patients treated with BMC injections for orthopedic conditions did not show any increase in new neoplasm rates [52].

There is a theoretical risk that injection of MSCs into or near tumor cells or malignancy could act to promote tumor growth and cell proliferation though this remains controversial [54].

Preoperative Considerations

- Patient needs to be aware of the following:
  - – Potential complications of BMA
    - Procedure site pain, infection, bleeding/hematoma, post-aspiration anemia, potential injury to surrounding structures, and embolic event in at-risk patients (cluneal nerves).
  - – Risks associated with intended target procedure (example: inadvertent dural puncture in disc procedure).
  - – Alternative treatments.
- Provider needs to be aware of the following:
  - – Pertinent medical issues or active infections that may increase procedural risk.
  - – Hematocrit levels should be assessed to estimate max BMA volume that can be harvested. For example, taking 60 mL of bone marrow aspirate in a small anemic female may be ill advised.

– Anti-coagulation status or bleeding disorders that could complicate normal clotting after penetration of the periosteum.
– If patient has a history of heparin-induced thrombocytopenia (HIT), then ACD (acid-citrate-dextrose) should be used to avoid blood clots during the aspiration.
• Provider needs to perform the following:
– Physical exam of the harvest area to assess for infection, skin ulcerations, or signs of injury.

Radiographic Guidance

Proper use of BMC in the treatment of musculoskeletal conditions requires image guidance both for precise administration of the injectate to the area of pathology, but also to perform a safe bone marrow aspiration that optimizes the amount and quality of MSCs obtained. Either fluoroscopy or ultrasound may be utilized, and both have their benefits and limitations. Attempting a BMA without imaging guidance is below the standard of care. Imaging guidance helps prevent significant complications from inappropriate trocar placement. The specific area of cannulation needs to be visualized to monitor cannula placement and to avoid areas of thin bone marrow cavity (Fig. 4.2).
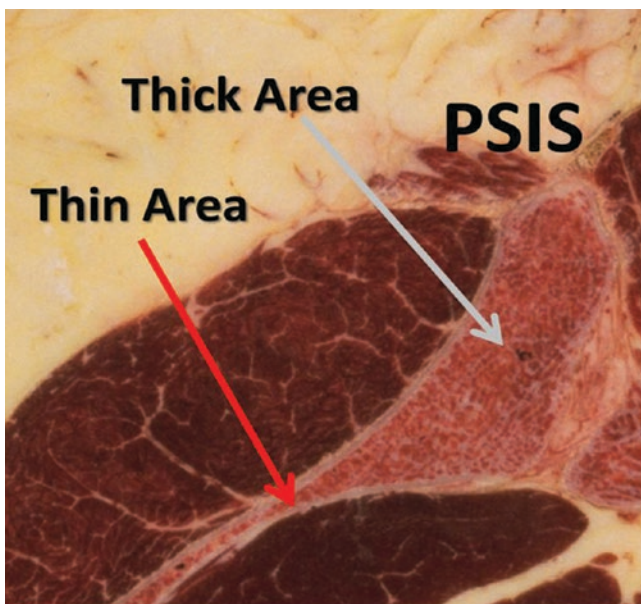

Fig. 4.2 A slice through the bony pelvis from the digital human project showing two marrow draw angles. The first through the "thin area" or the area identified as more radio-lucent. This is a thin area of the pelvis where the likelihood of passing through the marrow space is very high. The "thick area" noted here is the more radio-opaque area shown on the prior slide. This area has a large marrow space with less risk of passing through the marrow rich area and much higher likelihood of drawing whole marrow

Ultrasound

• PRO: Visualizing superficial and soft tissues as well as neurovascular structures.
• CON: Structures deep to bone are not able to be visualized.
• Example: Recommended to inject the rotator cuff of the shoulder, but not recommended when injecting the ACL of the knee due to the ligaments being inside the bony trochlear groove.

Fluoroscopy

• PRO: Visualizing bone and other deeper structures with the use of contrast.
• CON: Unable to image superficial soft tissues. Radiation exposure and cost.
• Example: Recommended for injecting stem cells into an osteonecrosis lesion of the hip, but would be less appropriate to inject a rotator cuff tear.

Key points to maximize MSC yield from BMC:

• The posterior superior iliac spine (PSIS) contains significantly more nucleated cells than other bone aspiration sites [55].
• Focus on drawing small volumes (5 mL per site) rather than drawing a large volume (over 20 cc) from a single bone site reduces [55].
• Multiple aspiration sites may yield more MSCs that reside in subcortical areas as well as pericytes that are located close to blood vessels.
• Ropivacaine 0.25% or less is *highly recommended* when providing local anesthesia. Any amount of bupivacaine or lidocaine can be toxic to MSCs [56, 57].

Equipment

• 30 g or 27 g needle
• 25 g 3.5-inch spinal needle
• Sterile 11-gauge disposable trocar (one for each side of access)
• 10–15 cc 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
• 5 cc syringe
• 30 cc syringes.[1]

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[1] Hernigou et al. suggested using multiple 5–10 cc syringes may increase MSC yield [58].

Technique

Harvesting Risk

Using the following bone marrow aspiration procedure guidelines, BMA is a safe and reliable procedure. A large U.K. registry reported an incidence of serious adverse event rate of 15 in a total of 20,323 procedures [59].

The steps for a BMA are as follows:

- The patient is positioned prone on a procedure table.
- After sterile prep, the skin is anesthetized with 10–15 mL local anesthetic. Ropivacaine 0.25% is highly recommended. If 1% Lidocaine is used, make certain that it does not contact the BMA.
  - Imaging guidance is critical during the injection of anesthetic. The skin, surrounding soft tissues, and periosteum need to be adequately anesthetized. If not, the patient may experience significant discomfort.
- After anesthetizing the skin and deep tissues, focus on drawing up the remaining medications to allow sufficient time for the local anesthetic to take effect.
- Draw 1 cc of 5000 IU/cc heparin into 5 cc syringe, and dilute it with an additional 4 cc of preservative free normal saline to make a 1000 IU/cc concentration (or follow the instructions of the point of care automated centrifuge).
- Draw 30,000 units into each 30 cc syringes intended for use, with a remaining concentration of 10,000 IU/cc.
- See Figs. 4.3, 4.4, and 4.5 to help guide angle of entry depending on imaging modality used. A shallow angle is used when using ultrasound (Fig. 4.3), and a steeper angle is used when using fluoroscopy (Fig. 4.4). Using these angles when approaching the PSIS (Fig. 4.5) optimizes draw sites where the bone marrow is best accessed in the safest fashion.
- Pass the trocar through anesthetized skin and soft tissues until contact is made at the bone cortex. Forward pressure is used while the device is turned clockwise/counterclockwise at the trocar handle, using the angled tip to bore a hole in the bone. Advancing another 5–10 mm will help seat the trocar in the cortex. The trocar may have incremental measurements to help gauge depth.
- Ensure the trocar is properly seated in the bone by wiggling the trocar handle gently. If it feels loose, further advancement will be needed, no more than 1 cm at a time and reassessing with another wiggle test. If the trocar resists any movement, no further advancement is needed.
- Remove the stylet from the trocar, and ensure the trocar is still well seated. Re-inserting the stylet and further advancing 1 cm at a time are not uncommon until adequate depth is achieved.
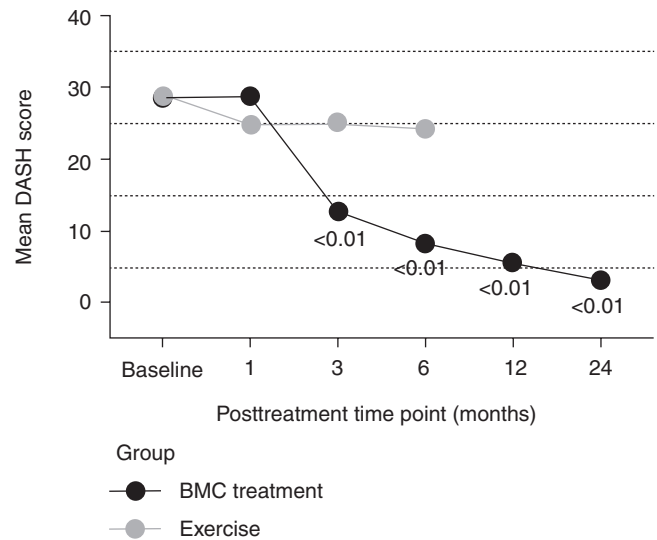

Fig. 4.3 Published randomized controlled trial results of rotator cuff tears treated with bone marrow concentrate injection versus exercise therapy
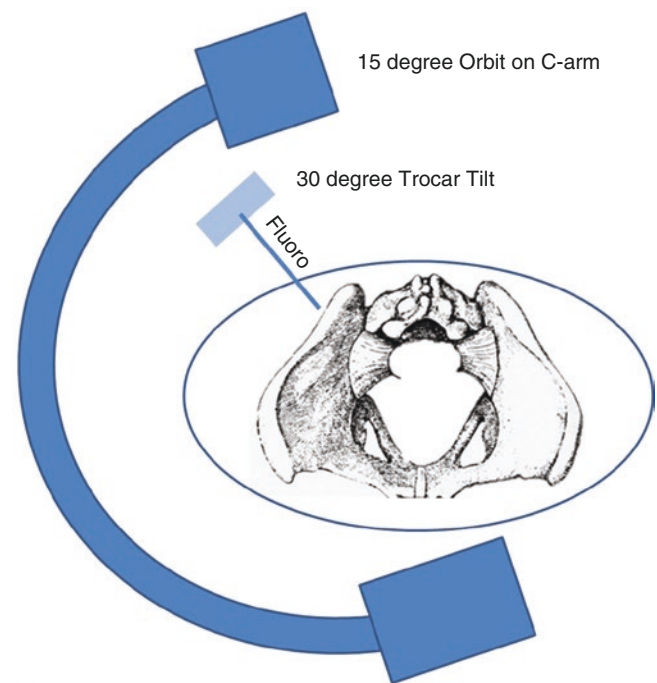

Fig. 4.4 Illustration of the ideal fluoroscopic C-arm and trocar placement for identification of the posterior superior iliac spine during a bone marrow aspiration procedure

- After the stylet is removed, attach the 5 cc syringe with 1000 IU/cc heparin and inject approximately 500–750 units to help prevent clotting. This step is important to prevent MSC trapping within a potential clot. This is performed for each bone site entered.
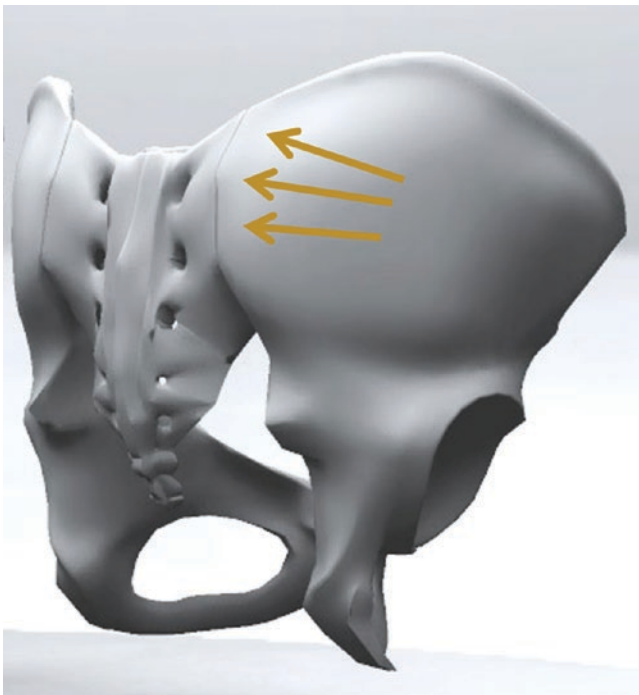
Fig. 4.5 Depiction of the posterior superior iliac spine (PSIS) located on the posterior pelvis. This is the ideal area for 3–4 draw sites from each PSIS

Table 4.5 A comparison between commercial bedside centrifuges and manual processing used to process bone marrow aspirate

	Commercial bedside centrifuge (510 K Approved)	Manual processing
Pros	Easy to learn Easy to use Lower start-up costs	Precise volumes, concentrations, cell counts Variable injectates are possible
Cons	Little versatility, variability Imprecise treatment protocols	Requires biologic safety cabinet Higher start-up costs More training required

- Attach the 30 cc draw syringe to the trocar. Pull back on the plunger according to patient tolerance. As BMA enters the syringe, gently agitate the syringe to help mix the heparin with the BMA to help mix the heparin and prevent clotting.
- Restrict the draw to 5–15 cc per site. Pull back and redirect the trocar without removing the trocar from the skin and reengage another bone cortex site. Any redirection needs to be performed under ultrasound or fluoroscopic guidance.
- Patient weight, number, and size of areas to be treated all help to determine the total BMA volume.
  - Females <47 kg, total volume should not exceed 50 cc.
  - Females >47 kg pounds but <54 kg, total volume should not exceed 60 cc.
  - Males or females >54 kg but <68 kg, total volume should not exceed 90 cc.

  - Male >68 kg, total volume should not exceed 120 cc.

Processing

The goal of BMA concentration is to isolate the buffy coat: the small, gray, middle section in a centrifuged BMA sample. Most providers injecting BMC utilize a commercial bedside centrifuge to concentrate the buffy coat rather than manual processing and lab technicians. There is limited third-party research available comparing these concentration devices. Table 4.5 helps describe the positive and negative aspects of each technique that are known.

510 K Approved Bedside Centrifuge Systems:

- Accelerate: Autologous Platelet Concentrating System
- Accelerated Biologics: BC 60 and BC 120 Pure
- Arthrex Angel
- BioCUE by Biomet
- Celling ART BMC
- CellPoint-ISTO Biologics
- Emcyte 544E
- Emcyte PureBMC
- GenesisCS Component Concentrating System
- Harvest Technologies SmartPrep 2
- ISTO CellPoint.

Dosing Bone Marrow Concentrate

Dosing of BMC can be quantified as follows:

- Colony-forming unit (CFU) assay: BMC is cultured in monolayer and incubated until colonies of MSCs form that adhere to plastic. The total number provides a rough metric of MSC content [60].
  - CFUs are primarily useful in the research setting rather than clinical, as the time needed for cell culture testing is not conducive to clinical practice setting.
- Flow cytometry: BMC cells are stained with fluorescent antibodies to MSC specific cell surface markers and processed through a flow cytometer. The International Society for Cellular Therapy issued a position statement, defining minimal criteria to identify an MSC. MSCs must express CD105, CD73, and CD90, but not CD34, CD45, CD14, CD11b, CD79alpha, CD19, or HLA-DR [61].
  - The cost and expertise required to run and analyze the results also makes this impractical in most clinic settings.
- Total Nucleated Cell (TNC) Count: the number of nucleated cells in BMC can be used as an indirect measurement, or proxy, of MSC content given the MSC/TNC ratio discussed above (0.01–0.5% of nucleated cells are MSCs).

– TNC is most convenient for clinical use. A manual hemocytometer or a commercial automated counting system is required (Peters and Watts 2016).

Research shows that better clinical outcomes is associated with higher CFU or TNC counts [11].

Post-operative Considerations

There are several medications known to impair MSC function and viability, and ultimately alter cell culture results. It is recommended that the following medications should be held for 2–3 serum half-lives before and at least 2–4 weeks after a BMC procedure to optimize clinical outcomes:

- Non-steroidal Anti-Inflammatories (NSAIDs) [62]
- Corticosteroids [63]
- ACE Inhibitors [64]
- Statins [65].

Potential Complications and Pitfalls

- Several local anesthetics, including Marcaine, Bupivacaine, and Lidocaine, are toxic to MSCs at low concentrations, and therefore, administering these in conjunction with BMC will significantly reduce cell viability. Ropivacaine at low concentrations of 0.125–0.25% is safe to use with MSCs [56, 57].
- It is very important to anesthetize not only the skin and subcutaneous soft tissue but also the periosteum. Incomplete anesthesia of the periosteum can lead to intense pain and even neuralgia.
- The clinician MUST provide adequate time for the local anesthetic to take effect (typically 3–5 minutes) prior to starting the procedure.
- BMAs using single site draws/collections with high volume aspiration (60 cc or more) will dramatically reduce cellular yield (please see Sect. 4.8).
- Preventing clots in the bone marrow aspirate sample is important to optimize cellular yield. Thus, it is imperative to pre-heparinizing the syringes used for sample collection as well as using heparin at the draw sites (the authors suggest using heparin—more effective anti-coagulant than ACD (anticoagulant citrate dextrose)).
  - Heparin must be used in the BMA draw syringe (see above) and should be gently shaken/mixed with the first BMA sample as soon as aspirated (it will not efficiently mix through diffusion).
  - During draw, immediately inject small amount of heparin (500–750 units) immediately after cannulating the cortex AND after each advancement of the trocar prior to aspirating.

Clinical Pearls

- It is important to remember that adipose tissue does not necessarily yield higher counts of stem cells.
- Forming a standardized routine is essential to proper BMA and patient comfort/safety.
  - Start with injecting local anesthetic to soft tissue and periosteum, step away and heparinize syringes, prepare trocar, set up image guidance, mark skin boundaries, etc. prior to starting the procedure.
- Remember to identify key anatomic landmarks when performing with fluoroscopy, prior to the procedure, to define target area.
- When using ultrasound guidance for imaging, the authors suggest using a sterile surgical marker on the skin to define safe borders for aspiration as well as to mark the previously anesthetized areas and prior draw sites.
- Use of a multi-site draw technique with several smaller aspiration volumes at each site will allow for higher cell yields.

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