

Bone Marrow Aspiration Technique, Concentration Methods and Applications

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OVERVIEW

The technique of bone marrow aspiration has evolved with the availability of improved designs of collection devices (e.g., Jamshidi needle, etc.) and aids for applying suction, including “VacLok®” syringes and constant vacuum-based aspiration. However, the protocol provided in this White Paper is based on the use of a manual aspiration technique, and is optimally applied with a 10 cc volume syringe, without the specific use of a “VacLok®” design. However, with some syringe designs, it is possible to “lock” the plunger in the fully extended position or the plunger position can be maintained by hand.

At the tissue level, it is important to apply sufficient “accelerative” force to engage the viscous bone marrow so as to enable its collection. This is achieved only by a very rapid pull-back on the plunger of the 10 cc syringe, which is not achievable with a 60 cc syringe. To be clear, it is not the total pressure change achieved during an aspiration cycle, but rather the change in pressure over time—what I am referring to as an “acceleration” of the plunger—that establishes a mechanical coupling of the suction with the red bone marrow and results in successful aspiration of high quality bone marrow. The accelerative force needs to be “high”, meaning that the application of the pressure gradient needs to occur over as short an interval as possible, in order to maximize the progenitor cell content of the collected aspirate. Implementing this approach means constantly pulling the plunger of a “fresh” collection syringe after collecting 2-7cc of bone marrow in the active syringe.

I have used the protocol outlined below to collect bone marrow aspirate that has very high “MSC” content.

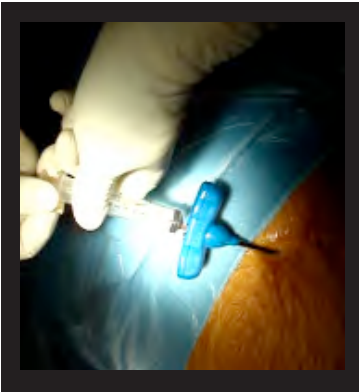
BONE MARROW COLLECTION

Collection of bone marrow from the iliac crest is accomplished by the use of a single beveled aspirating needle. The aspiration needle should have an inner diameter of 1.5 mm and a length of between 6 and 8 cm. The aspiration needle outer diameter should be as thin as possible, but should not exceed 4 mm. The technique works best with a single opening needle, but needles with 4-6 openings also will work. A standard 10-cc syringe should be used to obtain the bone marrow aspirate (BMA).

Preparation of the needle and syringe prior to insertion involves the rinsing of the needle and aspiration syringe with a heparin solution (2 mL heparin at 5000 units/mL, diluted in a container of 500 mL of sterile saline). This is accomplished by aspirating approximately 10 mL of the heparin solution into the syringe barrel. Heparin rinsing should be performed immediately (less than 2 minutes, but no longer than 4 minutes) before the needle/syringe is used for aspiration. Each replacement syringe also should be rinsed with heparin solution just prior to use.

Position the patient to permit access to the iliac crest (IC). Bone marrow can be collected from either the anterior or posterior part of the iliac crest in order to remain well within the tables of the crest. For a supine patient the target will be the anterior part of the IC, for a patient in a lateral position then the target will be either anterior or posterior and if the patient is face down then the posterior part of the IC will be the target. 2-3 channels (i.e., needle insertions) can be made in the anterior IC, and 3-4 channels can be made in the posterior IC. The number of channels used will depend on the total amount of bone marrow aspirate required for the application and the yield obtained from the first aspiration channel.

For the normal weight patients a direct puncture with the needle is appropriate, but if the patient is obese a 2 mm incision should be made at the site of collection. Insert the beveled, aspiration needle 6 cm into the cancellous bone of the IC (spongy bone), creating the first channel. Pull back on the syringe plunger as rapidly as possible to initiate collection of the BMA—maintaining displacement of the plunger during the aspiration process. After collecting 2-4 cc (up to 7cc) of bone marrow, detach the syringe (without releasing the pressure), hand off the filled syringe and attach a fresh syringe. Prior to pulling back on the plunger and without changing depth of the needle point, turn the syringe needle 45° and then rapidly pull on the plunger. Repeat this process a total of 8 times (360°) without changing the depth of the needle point, with the bevel being rotated 45° each time.

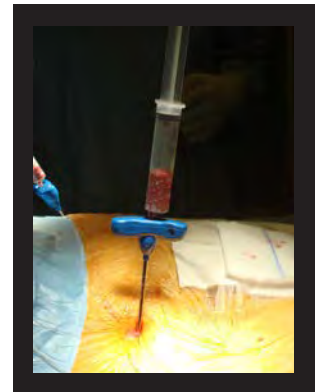


It is important to understand that you should attempt to perform one aspiration cycle per position of the bevel. During an aspiration attempt, the following situations may occur:

- No bone marrow is aspirated. Turn the bevel 45° and repeat the aspiration process until bone marrow is aspirated.
- 1-2 cc is collected. Turn the syringe 45° and re-pull the plunger without switching the syringe.
- Rich bone marrow is collected—as much as 6-7 cc can accumulate in one syringe before switching the syringe.
- If the first position of aspiration in a channel is “dry” or has a low yield (<2cc), the first position should be re-aspirated after completing the rotation at that level.

For maximum recovery of progenitor cells, collecting 2 cc per bevel position at a single level in a channel is acceptable. Depending on the richness of the bone marrow deposit, up to 7cc per bevel position may be collected. After collecting BMA at the lowest level of the first channel, pull the needle point toward the surface by 1-2 cm. Repeat the process of 2-4 cc aspirate collection per bevel position at the second level.

Continue the BM collection process in the initial channel until the bevel of the needle is located within 1 cm of the outer edge of the IC. Expect to collect as much as 32 cc of BMA at each level per channel, giving a total of between 48 and 96 cc per channel (depending on whether 2 or 4 cc of BMA are collected per bevel position and if up to 3 levels have been collected within a channel).



Collection from multiple insertion points will follow the curvature of the IC. Ensure that the needle point creates a channel that is parallel to previous channels in order to avoid pulling blood into the collection syringe. Approximately 100 cc is the largest volume of BMA that should be collected from the first channel. Subsequent BMA collection volumes from adjacent channels in the anterior IC typically will be lower, which supports a limit of 150 cc as the maximum volume of BMA that should be collected from the anterior IC.



Due to the larger volume of bone marrow typically present in the posterior IC, it is possible to collect as much as 180-200 cc in the first channel, with a maximum collection volume of 250 cc in the posterior IC. Thus, for a bilateral collection, it is possible to obtain as much as 500 cc of BMA. Pool the aspirates as quickly as possible in a collection container in which ACD-A already is present at an anticipated ratio of 1:10 (v/v, ACD-A:BMA). The collection container also should have 1-2 mL of the heparin solution described above per 100 cc of BMA to be collected. Good mixing is important for preventing clot formation.

CONCENTRATION METHODS

Concentration of the bone marrow aspirate is necessary to maximize the therapeutic benefit of the process. Concentration refers not just to reducing the volume of the cell preparation, but to eliminating RBCs. Ultimately, shifting the composition of the bone marrow concentrate (BMC) toward the mononuclear cell type will provide cells with a more “regenerative” potential in a smaller volume. One such device that has been used to achieve this is the COBE instrument. A single “cycle” through the COBE results in a concentration of 5-7X, while a second treatment cycle results in approximately a 10X concentration over the starting BMA. Further concentration is not practical. Two cycles of concentration are needed when treating small lesions, while a single cycle is sufficient for treating larger lesions. Another processing option is to use bone marrow concentration technology, like the ART 21 system (SpineSmith Partners, Austin, TX). In this system 60 cc of bone marrow aspirate is loaded into a disposable device and centrifuged. The BMC is obtained from the post-centrifuged device in a volume ranging from 3.5 to 10 cc, giving the option of obtaining concentrations of BMC in excess of 10X. Another technique for RBC reduction and mononuclear cell enrichment is the use of Ficoll-Paque.

PATIENT FACTORS AFFECTING BONE MARROW COLLECTION

There are obvious differences in the volume of bone marrow among patients, but the physical dimensions of the iliac crest don't necessarily correlate with the “goodness” of a collection. One source of variation is the portion of the marrow that is “red” or “yellow”. “Red” bone marrow is the best source of progenitor cells. However, during collection it is difficult to see the difference between these two types of bone marrow. On a practical level, generally speaking, yellow marrow will not yield more than 1-2 cc in volume, so the collection should be stopped, the bevel repositioned by 45° and a new vacuum applied by rapidly pulling back on the syringe plunger. Other factors that can affect the volume of bone marrow collection include: a patient who smokes; a woman going through her menstrual cycle; changes in temperature (which affects the distribution of RBCs in the extremities); and the age of the patient, with young adults having the highest progenitor cell levels.

APPLICATIONS OF BM CONCENTRATE

Avascular Necrosis in the Femoral Head

Bone marrow concentrate is injected into the femoral head using a small trephine (e.g., trocar of Mazabraud, Collin, France). The instrument is introduced through the greater trochanter, as in conventional core decompression. Its position in the femoral head and in the necrotic segment is monitored with biplane fluoroscopy. Since, at the time of treatment, the plain radiographs will show little if any evidence of necrosis, the preoperative MRI scans should be used together with the image intensifier views, to determine the site of the lesion. After injection of the bone marrow concentrate, a few mL of contrast medium should be injected, in order to check the spread of the BMC in the area of the femoral head. It has been established that the contrast medium will not damage the bone marrow progenitor cells.

Although the bore of the trocar is small compared with the trephines normally used for core decompression, femoral head and trochanteric region pressure measurements have shown that even a small hole will relieve the intraosseous pressure. During the bone marrow concentrate injection the pressure in the femoral head will rise, but a normal pressure pattern is restored after completing the injection, exactly as in intraosseous pressure measurements. In our patients, no complications were observed during anaesthesia; in particular, there is no reduction in oxygen saturation, and no change in the pulse rate or blood pressure.

Volume of Injection:

Estimate the volume of the necrotic zone by MRI and determine the shape of the area to be treated. For large

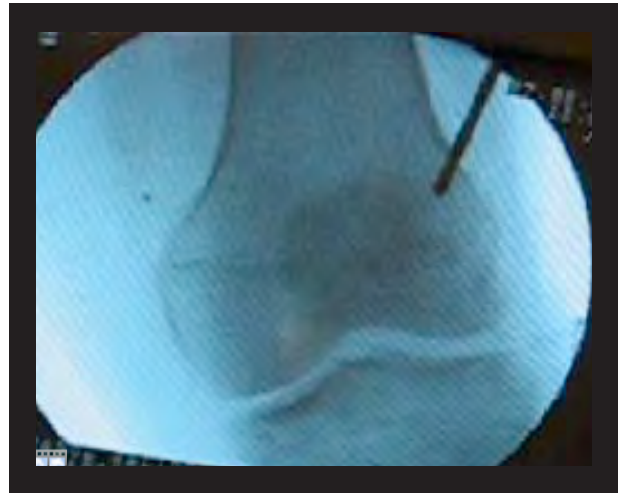
volume AVN approximately 20 cc of concentrated cell preparation will be injected, with a range of injected volumes of 15-25 cc. Between a minimum of 10-15 cc of BMC should be injected into the channel, with the remainder being injected into the adjacent bony structure.

Composition of Injection:

BMC without any alterations, so the “carrier” is the plasma isolated from the patient.

Injection options:

The bone marrow aspirate is collected prior to the start of the procedure, collecting approximately 150 cc of BMA for a large femoral head AVN. The initial step in treating AVN in the femoral head is to do a core decompression. Inject the BMC slowly into the channel to allow the cell preparation to diffuse into adjacent tissue. Additional BMC should be introduced into the areas immediately outside of the necrotic section once the necrotic zone has been treated. If there is resistance in dispensing the BMC into the channel, pull back on the trocar 5-10mm and then inject. This approach creates a small chamber, aiding the dispersal of the BMC into the channel.



NON-UNION FRACTURE TREATMENT

The same trocar as is used to aspirate the marrow is placed in the non-union gap and around the bone ends. The position of the tip of the trocar is monitored with biplane fluoroscopy. The trocar site perforation on the leg should not be adjacent to tendons or major neurovascular structures and the trocar should be pushed directly to the surface of the bone. For a tibial fracture, the skin perforation is done on the lateral part of the anterior tibial crest. Through the same skin perforation it is possible to position the trocar in the non-union site and on the medial, lateral and posterior parts of the non-union. Bone marrow concentrate is injected slowly at a rate of about 20 mL/min with a 10 mL syringe. The volume of bone marrow graft injected in the non-union is 20 mL. In some cases, the pressure required to inject BMC is high and leakage may occur through the trephine site; in such a circumstance, it is necessary to change the position of the tip of the trocar. After injection, the trocar, with the stylet in place, is gradually withdrawn with small oscillating motions (backward and forward) to fill the path of the trocar. The perforation in the skin is closed with a circumferential suture to avoid leakage of marrow.

Volume of Injection:

The volume of injection to treat a non-union will depend on the age of the patient, with young pediatric patients (\leq 10 yrs old) receiving no more than 10 cc of BMC and older patients receiving up to 20 cc.

Composition of Injection:

For percutaneous injections, the BMC can be directly injected without “additives” or the BMC can be combined with a granular (e.g., 1-2 mm particles) bone void filler, which has been mixed with the BMC for several minutes prior to injection. In an open surgical procedure, a ceramic block may be used to fill the gap. In this case, it is best to place

the ceramic implant into the gap and then fill the ceramic by slowly injecting the BMC into the interior of the ceramic implant, ensuring the spreading of the BMC suspension. The size of the gap in the non-union will dictate the surgical approach, such that gaps greater than 2 cm typically are filled by ceramic implants and then the BMC is injected. BMC mixed with a granular bone void filler also can be injected into the ceramic implant.

Injection options:

The injection should be directed into the fibrous non-bony tissue in the non-union gap, under fluoroscopic visualization. Injection is done by inserting the needle in such a way that it is possible to inject at several locations along the needle channel, extending from the periphery through the interior of the non-union gap.

Soft Tissue-Bony Interfaces-Rotator Cuff Repair

Volume of Injection:

Inject 4 cc of BMC into the tendon (tangential approach) once the tendon has been fixed with the anchor. Look for swelling of the tendon thickness. If the rotator cuff tendon is large, then injection at two sites should be considered.

Inject 4-6 cc of BMC into the bone just at the bone/tendon interface. Use fibrin glue in a 4 cc portion of BMC in order to “coat” the surface of the bone-tendon interface. This is applied during a “dry” portion of the arthroscopic surgery. The cell preparation is allowed to clot, which allows for its adhesion to the bone-tendon interface even in an aqueous environment.

Inject 4 cc into the muscle tissue approximately 4 cm from the site of repair, literally aiming for the middle of the muscle. This is injected percutaneously.

Injection Composition:

The “serum” obtained during the processing of the BMA is used as the primary suspension fluid. However, fibrin glue is added in order to better coat the bone-tendon interface, especially during arthroscopic procedures.

Injection Options:

BMC volumes and locations remain the same whether the procedure is done arthroscopically or in an “open” procedure. However, during an open procedure it is not necessary to create a fibrin glue for coating the surface of the bone-tendon interface.

Enhancing Osteogenic Potential of Allograft for Arthroplasty Revision

The ideal bone graft should display mechanical properties similar to the bone recipient. Today, allogenic bone remains the most frequently used in hip revision due to these mechanical properties. However, most of the allografts are dead bone without any cells and without osteogenic capacity. They act as artificial materials due to their lack of osteoconductive properties.

The purpose of combining BMC with allograft is to enhance the osteogenic potential of the implanted allograft. The primary method is to combine 10 cc BMC with 10 cc of allograft, allowing the allograft to absorb the BMC. Once the BMC-loaded allograft is ready it can be placed or injected as described: the 10cc of bone marrow are injected through the articular surface of the femoral head with several points of injection distributed at the whole surface of the femoral head.