

Office-Based Harvest of Mesenchymal Stem Cells by Tibial Intraosseous Cannulation: Part I

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ABSTRACT

A recent development in regenerative medicine is the use of bone marrow derived mesenchymal stem cells for the treatment of degenerative musculoskeletal conditions such as osteoarthritis. While the use of autologous stem cells is of immediate interest to all practitioners of regenerative medicine, the complexity and expense of the traditional technique of harvesting bone marrow from the iliac crest with the use of a trocar presents obstacles for Prolotherapists and patients alike. The purpose of this two part article is to propose a novel technique intended to harvest and deliver bone marrow derived mesenchymal stem cells that is at the same time simple, safe, and inexpensive to perform.

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KEYWORDS: bone marrow, mesenchymal stem cells, Prolotherapy, stem cell therapy.

This is the first in a series of two articles investigating novel and inexpensive methods of harvesting and preparing bone marrow derived mesenchymal stem cells (MSC).

Prolotherapy is an alternative therapy for treating musculoskeletal pain that involves injecting an irritant substance into damaged connective tissues to promote the growth of new, healthy tissue.¹ Because Prolotherapy aims to induce the growth of tissues to repair or replace damaged cartilaginous structures, it falls into the category of “Regenerative Medicine”.² In recent years, many Prolotherapists have begun using platelet rich plasma (PRP) in place of irritant solutions to treat conditions such as osteoarthritis (OA) and tendinopathies.³ Because of its simplicity, safety, and affordability, PRP preparation and delivery is easily introduced into a Prolotherapy practice. A recent development in regenerative medicine is the use

of bone marrow derived mesenchymal stem cells for the treatment of degenerative musculoskeletal conditions such as osteoarthritis. While the use of autologous stem cells is of immediate interest to all practitioners of regenerative medicine, the complexity and expense of the traditional technique of harvesting bone marrow from the iliac crest with the use of a trocar presents obstacles for Prolotherapists and patients alike. The purpose of this article is to describe a novel technique to harvest and deliver bone marrow derived mesenchymal stem cells that is at the same time simple, safe, and inexpensive to perform.

Emerging data supports the percutaneous injection of bone marrow derived mesenchymal stem cells for the regeneration of articular cartilage. Saw, et al. found that postoperative intra-articular injections of autologous bone marrow in combination with hyaluronic acid after subchondral drilling resulted in better cartilage repair in a goat model.⁴ Fortier, et al. found that delivery of bone marrow concentrate resulted in healing of acute full-thickness cartilage defects that was superior to healing after microfracture alone in an equine model.⁵ Murphy, et al. found that local delivery of autologous MSC to injured joints stimulates regeneration of meniscal tissue in a goat model. In a case study, Centeno, et al. found that autologous MSC culture and percutaneous injection into a knee with symptomatic and radiographic degenerative joint disease resulted in significant cartilage growth, decreased pain, and increased joint mobility.⁶

Prolotherapy^{7, 8} and PRP⁹ have shown promise in the treatment of OA. The potential benefits of injecting MSC directly into degenerated joints are clear to any Prolotherapist; it could be considered the natural evolution of Prolotherapy. However, bone marrow aspiration has

traditionally been performed in a surgical suite using fluoroscopic guidance with either conscious sedation or general anesthesia making the procedure more complex than most Prolotherapy offices are equipped for. Iliac crest marrow is then concentrated using commercially available systems, rendering bone marrow aspirate concentrate (BMAC), which is then blended with PRP in order to provide the MSC with growth factors to promote growth and engraftment.¹⁰ The complexity of this procedure combined with the expense of the materials makes it cost prohibitive to many patients. Resultantly, this author has sought a method to harvest MSC for reinjection that is safe, simple, and inexpensive.

Intraosseous infusion (IO), a technique for vascular access, was first described in 1922 and was widely used for drug administration in pediatric medicine by the 1940s.¹¹ Its use declined during the 1950s with the advent of single-use intravenous catheters, but re-emerged in the 1980s¹² and today IO is widely used in emergency medicine both pre-hospital and as an alternative to the central line.¹³ A commercially available IO device is the EZ-IO® Intraosseous Infusion System. The EZ-IO is a small, battery-powered, handheld device that drives a beveled, hollow, drill-tipped needle set. It is designed to access the proximal humerus or the proximal or distal tibia. The EZ-IO provides the non-surgeon easy and rapid access to the intramedullary space and enjoys an excellent safety profile.¹⁴

This past year, this author has injected a blend of autologous bone marrow collected from the tibia using the EZ-IO and PRP into eight large peripheral joints of as many patients as well as into the dermis of the scalp of one patient for the treatment of male pattern baldness and into the dermis of the face of a second patient for the treatment of aging skin. Results are promising and no adverse effects were reported beyond injection discomfort and the initial soreness and stiffness normally associated with Prolotherapy.

To avoid intravascular injection of the high concentration of adipocytes found in long bone marrow, this author has limited this procedure to intra-articular injection of large joints (hip, knee, and shoulder) and superficial cosmetic injections in otherwise healthy patients.

Indications include damaged or degenerated articular surfaces of the shoulder, hip, and knee and damaged

cruciate ligaments of the knee. Based on data surrounding blind injection of peripheral joints, ultrasound guidance is used when injecting the hip and shoulder and is optional when injecting the knee.¹⁵ Cosmetic injections to the scalp and face are kept superficial to the hypodermis.

METHOD:

1. Prepare 4mL of PRP with 1mL of anticoagulant citrate dextrose (ACD) in a 10mL syringe (5 out of 10mL of syringe-space is occupied).
2. Identify harvest site—approximately 2cm distal to tibial plateau and 2cm medial to tibial tuberosity.
3. Prepare skin over harvest site.
4. Inject harvest site with 2mL of 2% lidocaine without epinephrine.
5. Position the needle and at a 90-degree angle to the surface of the bone (this will give a slight cephalad orientation due to curvature of the surface bone at this site). (See Figure 1.)



Figure 1. The drill-tipped needle pierces the skin.

6. Advance needle through skin until tip touches bone. (See Figure 2.)
7. Squeeze driver's trigger and apply steady downward pressure until a "pop" is felt upon entry into medullary space.
8. Remove power driver and stylet. (See Figures 3 & 4.)
9. Attach PRP syringe (with or without EZ-Connect Extension Set) to catheter (if using EZ-Connect Extension Set, flush with ACD prior to use).
10. Draw 5mL of aspirate into a 10mL syringe already containing 5mL of PRP.* (See Figure 5.)
11. Remove syringe and/or EZ-Connect Extension Set.
12. Remove catheter by rotating and pulling NOT by rocking or bending.



Figure 2. The drill-tipped needle is advanced through the trabeculae into the medullary space.



Figure 5. Bone marrow is then aspirated. Typically, 5mL is drawn into a 10mL syringe already containing 5mL of PRP.



Figure 3. The power driver (drill) is removed.



Figure 4. The stylet is removed.

13. Apply sterile pressure dressing.
14. Gently mix PRP and bone marrow blend.
15. Prepare skin over injection site.
16. Insert hypodermic needle into joint being treated.
17. Inject 1mL 8% procaine.** (See Figure 6.)



Figure 6. Procaine anesthetic is injected into the joint.

18. Leave needle in place and wait 30 seconds.
19. Attach PRP/bone marrow syringe to needle. (See Figure 7.)
20. Inject PRP/bone marrow into joint. (See Figure 8.)
21. Remove needle.
22. Have patient remain motionless for 1 hour to allow for cell attachment.†
23. Cephalexin 500mg by mouth twice per day for four days.††



Figure 7. Bone marrow aspirate syringe is attached to the needle hub.



Figure 8. Bone marrow is directly injected into the joint.

* Aspirate over 10mL from a single intramedullary site is peripheral blood only.

** Procaine is used because of known cytotoxic effects of lidocaine and bupivacaine on synovial tissue¹⁶ and an 8% concentration is used to maximize the effect of a small volume.

† After ten minutes approximately 60% of MSC attach.¹⁷

†† Common practice among our surgical colleagues is to administer prophylactic antibiotic therapy concomitant with bone marrow aspiration.

DISCUSSION:

This author does not seek to replace the gold standard, BMAC from the iliac crest, with the described method. Rather, because of its technical simplicity and low material expense, tibia aspiration is explored as an alternative treatment for those patients who are candidates for

percutaneous injection with autologous MSC and cannot afford BMAC. The described technique is easier and less expensive to perform than is BMAC from the iliac crest, thereby increasing economic feasibility. It is safe; at worst we are diluting PRP with whole blood and adipocytes drawn from the tibia. But does this method of tibia marrow aspiration hold the same promise for clinical effectiveness as BMAC from the iliac crest? The yellow marrow of the tibia contains approximately half the amount of MSC as does the red marrow of the iliac crest.^{18, 19} Therefore 10mL of aspirate from a single site in the tibia rather than 60-120mL from up to six sites from in iliac crest, at the very best, is providing approximately 1/12 the number of MSC. How important is concentration and number of MSC? In a clinical case series of sixty patients, Hernigou and his group found percutaneous autologous bone marrow grafting was an effective and safe method for the treatment of an atrophic tibial diaphyseal nonunion. However, its efficacy appears to be related to the number of progenitors in the graft, and the number of progenitors available in bone marrow aspirated from the iliac crest appears to be less than optimal in the absence of concentration.²⁰

An obvious improvement on this described method would be to draw a larger volume of tibia marrow and concentrate the MSC with centrifugation. Because of the experimental nature of this procedure, I have not yet felt justified to perform multiple tibia cortical punctures in a single encounter, thereby limiting the aspirate volume to 10mL. My rationale for not concentrating tibial marrow at this early stage is because of the uncertainty of how centrifugation would affect delivery of MSC. Tibia marrow has a high volume of adipocytes compared to iliac crest and MSC are highly adherent.²¹ Subsequently, there is potential for the MSC to adhere to the adipocytes and thereby be lost during centrifugation. The adherent nature of MSC presents an additional pitfall: the EZ-IO is designed for rapid delivery of emergency medicines and fluids, not for marrow aspiration, and therefore the needle has only a single, large port. This may disallow for the creation of sufficient negative pressure to pull MSC free from the tissue mass. MSC harvest from the tibia could be improved by the creation of a specialized tibia marrow instrument that is longer to allow for repositioning for multiple aspiration sites with a single cortex puncture and includes a multi-port, fenestrated catheter and a Jamshidi-style needle for bone coring. Above all, laboratory investigation into best-

practice tibia marrow MSC preparation is required to determine if tibia marrow aspiration holds promise.

This author seeks to find a safe, simple and affordable method for MSC harvest and presents the concept of tibia marrow collection in its earliest stages in hopes of creating interest among colleagues for collaboration into further investigation. This author welcomes communication from the Prolotherapy and surgical communities.

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BIOLOGRAPHY

1. Definition of Prolotherapy – www.merriam-webster.com.
2. Regenerative Medicine Fact Sheet, Nation Institute for Health, 2006.
3. Crane D, et al. Platelet rich plasma matrix grafts. *Practical Pain Management*. Volume 8 Issue 1; January/February 2008; pp12-26.
4. Saw KY, et al. Articular cartilage regeneration with autologous marrow aspirate and hyaluronic acid: an experimental study in a goat model. *Arthroscopy*. 2009 Dec;25(12):1391-400.
5. Fortier LA, et al. Concentrated bone marrow aspirate improves full-thickness cartilage repair compared with microfracture in the equine model. *The Journal of Bone and Joint Surgery (American)*. 2010;92:1927-1937.
6. Centeno CJ et al. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician*. 2008; 11:3:343-353.
7. Reeves KD, et al. Randomized prospective double-blind placebo-controlled study of dextrose Prolotherapy for knee osteoarthritis with or without ACL laxity. *Alt Ther Hlth Med*. 2000;6(2):37-46.
8. Reeves KD, et al. Randomized prospective placebo controlled double blind study of dextrose Prolotherapy for osteoarthritic thumbs and finger (DIP, PIP and Trapeziometacarpal) joints: Evidence of Clinical Efficacy. *Jnl Alt Compl Med*. 2000;6(4):311-320.
9. Kon E, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc*. 2010 Apr;18(4):472-479.
10. Centeno CJ, et al. Increased knee cartilage volume in degenerative joint disease using percutaneously implanted, autologous mesenchymal stem cells. *Pain Physician*. 2008; 11:3:343-353.
11. Foex BA. Discovery of the intraosseous route for fluid administration. *J Accid Emerg Med*. 2000;17:136.
12. Fiser DH. Intraosseous infusion. *N Engl J Med*. 1990;322:1579.
13. Paxton JH, et al. Proximal humerus intraosseous infusion: a preferred emergency venous access. *J Trauma*. Sep 2009;67(3):606-11.
14. Ngo AS, et al. Intraosseous vascular access in adults using the EZ-IO in an emergency department. *Int J Emerg Med*. 2009;2:155-160.
15. Lopes RV. Accuracy of intra-articular injections in peripheral joints performed blindly in patients with rheumatoid arthritis. *Rheumatology*. 2008;47(12):1792-1794.
16. Bogatch MT, et al. Is chemical incompatibility responsible for chondrocyte death induced by local anesthetics? *Am J Sports Med*. 2010;Mar;38(3):520-526.
17. Koga H, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther*. 2008;10(4):R84.
18. Marx R, et al. 2006 Unpublished data of ten consecutive bone marrow aspirates taken from patients iliac crest and proximal tibia and comparing the nucleated cell count, cell smears and flow cytometry analysis.
19. Schon L, et al. 2007 Unpublished data – Osteoprogenitor cells were more abundant in iliac crest than in the Calcaneus (p<0.002) and the tibia (p<0.015).
20. Hernigou P, et al. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *Bone Joint Surg Am*. 2005; Jul;87(7):1430-7.
21. Jäger M, et al. Cell therapy in bone-healing disorders. *Orthopade*. 2010;Apr;39(4):449-462.