INTERNATIONAL JOURNAL OF ORTHOPLASTIC SURGERY

Miller, TJ, et al. Regenerative Surgery & Intra-Operative Protocols Utilizing Bone Marrow Aspirate Concentrate in Microsurgical & Limb Reconstruction. *International Journal of Orthoplastic Surgery.* 2019; 2(2), pp. 39–46. DOI: https://doi.org/10.29337/ijops.29

ORIGINAL RESEARCH

Regenerative Surgery & Intra-Operative Protocols Utilizing Bone Marrow Aspirate Concentrate in Microsurgical & Limb Reconstruction

Timothy J. Miller*, Edgardo Rodriguez-Collazo†, Stephen J. Frania* and Alessandro Thione‡,§

Regenerative medicine is a resource to restore structure and function to damaged tissues and organs, and to support the body's own healing mechanisms. For surgeons, intraoperative cell therapies that integrate autologous cell-based therapy with surgical interventions are aiding patients in their own recovery. Intraoperative cell therapy includes tissue harvesting and processing in combination with surgical techniques and the cell-derivative application. The purpose of this paper is to provide the technique for obtaining bone marrow aspirate during surgery, and it's applications in surgical interventions that include bone lengthening and arthrodesis, muscular transpositions, and micro peripheral nerve reconstruction.

Keywords: bone marrow; aspirate; regenerative medicine

Introduction

Regenerative medicine is a resource to restore structure and function to damaged tissues and organs, and to support the body's own healing mechanisms. For surgeons, intraoperative cell therapies which integrate autologous cell-based therapy with surgical interventions are aiding patients in their own recovery. Intraoperative cell therapy includes tissue harvesting and processing in combination with surgical techniques and cell-derivative application [1]. Advanced regenerative medicine strategies, in combination with reconstructive techniques, allow physicians to combine approaches and facilitate their efforts in complex deformities. Concentrated bone marrow aspirate (cBMA) is a regenerative therapy where peripheral blood from the bone marrow is taken from the patient and is effectively filtered and centrifuged into a concentrate containing a higher ratio of stem cells, including mesenchymal stem cells (MSCs) and hematopoetic stem cells (HSCs) [3]. Procedures where cBMA is being used in microsurgical and reconstructive surgeries are bone lengthening and arthrodesis, pedicle vascularized muscular transpositions, and micro peripheral nerve reconstruction [10]. Today, point of care devices for the creation of autologous cellular therapies rich in stem cells and growth factors provide easy options for clinical utilization. The use of these devices is certainly logical and practical given the documented ability of their output in improving healing. Platelet rich plasma (PRP) also has benefits. Platelets release antimicrobial molecules by engaging bacterial pathogens specifically and form the adaptive immunity [2]. PRP promotes tissue regeneration, enhances collagen synthesis, and triggers angiogenesis and immune responses by releasing growth factors and cytokines [2]. PRP has also been shown to increase MSC proliferation as well [8]. The purpose of this paper is to provide the technique for obtaining bone marrow aspirate during surgery, and it's applications in surgical interventions that include bone lengthening and arthrodesis, muscular transpositions, and micro peripheral nerve reconstruction.

Operative Technique

Preoperatively, 52 mL of peripheral blood is drawn into a syringe containing 8 mL anticoagulant citrate dextrose solution as seen in **Figure 1** (ACD-A, Isto Biologics, Hopkinton, Mass.) The 60 mL of the anticoagulated blood is placed in the Magellan System (Isto Biologics, Hopkinton, MA) to obtain 10 mL of platelet rich plasma (PRP) and approximately 12 mL of platelet-poor plasma (PPP), as seen in **Table 1, Figure 2**.

Intraoperatively, the tibial tuberosity is palpated of the limb where the bone marrow will be obtained. Next, the anterior crest of the tibia, the postero-medial border of the tibia, and the tibial tuberosity are marked. Measurements are taken 1 cm distal to the tibial tuberosity. Then, find an area in the center of the tibia

* Department of Surgery-Lake Health System, Mentor, OH, US

† Department of Surgery-Amita Health Saint Joseph Hospital, Chicago IL, US

‡ Department of Surgery-University of Insubria, ES

§ Plastic Surgery and Burn Unit, Hospital la Fe, Valencia, ES

Corresponding author: Alessandro Thione, MD, PhD (althione@gmail.com)

(marked from the anterior crest and postero-medial border), approximately 1.5 cm medial to the anterior tibial crest (**Figure 3**). At this portion, a stab incision is made with blunt dissection continued deep to include the periosteum. A 15 gauge 6-port fenestrated jamshidi is inserted into the surgical site, placed perpendicular to the medial surface. A mallet is used to help the trocar penetrate the cortical bone so that the needle can access the marrow cavity. The mallet is used until you feel a loss of resistance as the needle "sinks" in. Care is taken to make sure that the perforation into the medullary canal is as small as possible because the ability to maintain negative pressure is crucial in obtaining the aspirate without drawing unwanted peripheral blood. The trocar is then removed from the cannula apparatus. Two 30 mL syringes are used to extract the bone marrow

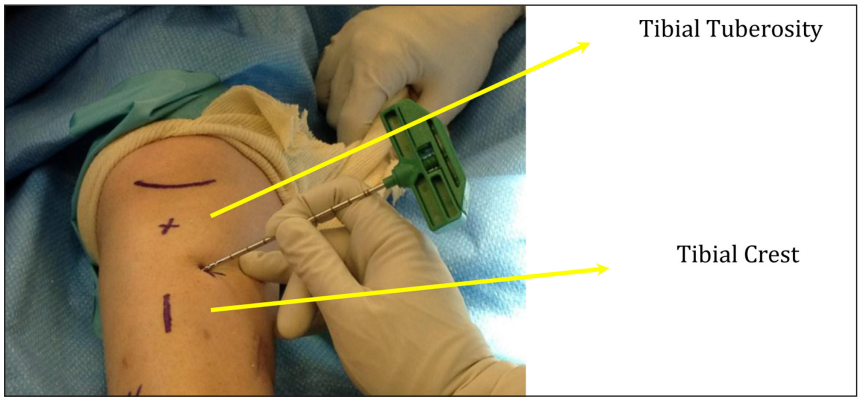
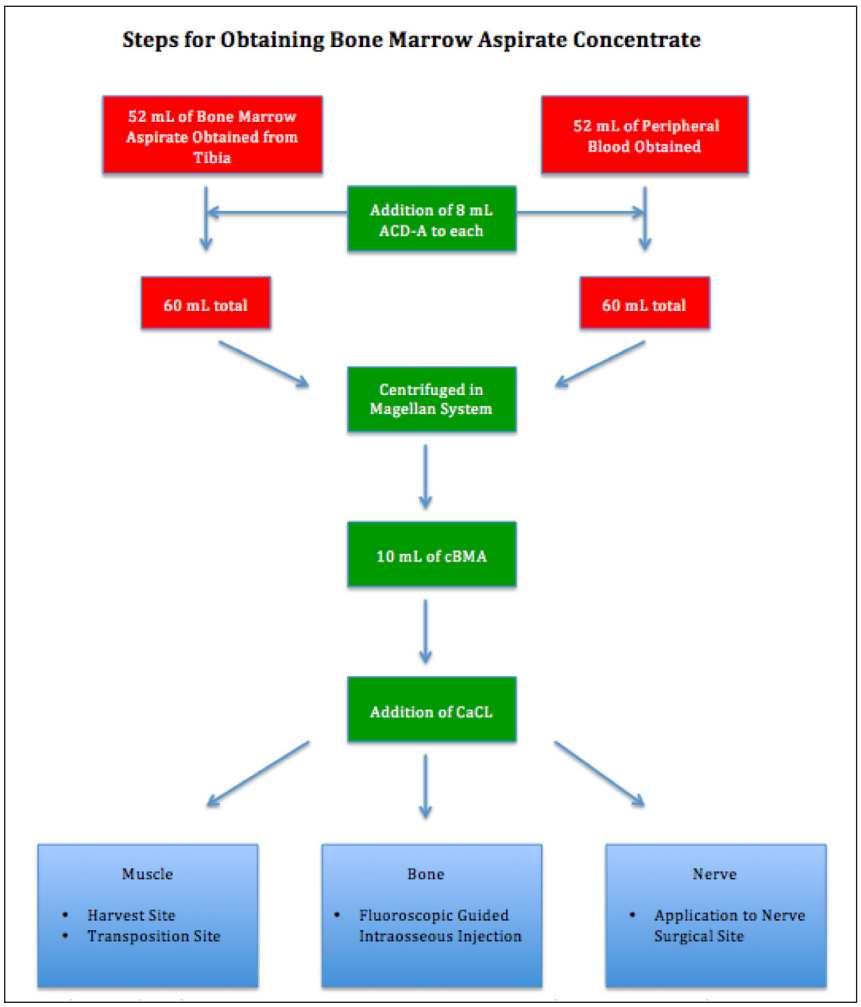


Figure 1: Alignment of needle on tibia for cBMA obtainment showing the tibial tuberosity and medial border of the tibia. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

Table 1: Steps for Obtaining Bone Marrow Aspirate Concentrate.



Credit: Timothy Miller.

aspirate. Slow aspiration of the bone marrow aspirate is performed to prevent lysis of the cells (**Figure 4**). Fifty-two mL of bone marrow aspirate is drawn in total with 8 mL ACD-A under negative pressure. After filtration, the BMA is placed in the Magellan System to obtain 10 mL of cBMA. The cBMA, PRP and PPP are all combined with calcified thrombin (5.000 units/5 mL of CaCl2) at application (**Table 1**). Additional BMA can be acquired by first removing the syringe from the aspiration portal and then reinserting the trocar and withdrawing the needle just enough to redirect it 25° in either direction from the original aspiration site (i.e., "fan" technique). When the needle is repositioned, the trocar is removed, and the syringe is reattached to aspirate the additional bone marrow. Once the desired amount is achieved, the trocar is inserted back into the cannula, and the apparatus is removed in its entirety. Pressure is applied to surgical site, and surgical site is closed primarily with surgeons' choice.

Summary for Operative Technique:

- 52 mL of peripheral blood centrifuged down
- 52 mL of bone marrow aspirate centrifuged down
- Measurements in tibia: 1 cm distal to tibial tuberosity, 1.5 cm medial to anterior tibial crest
- Needle position perpendicular to cortical surface
- Fan technique can be used if more aspirate is needed

Protocols for Bone Lengthening and Arthrodesis

The cBMA sample contains, among many other regenerative cells, mesenchymal stem cells (MSCs). These cells exhibit multipotent differentiation potential, and have

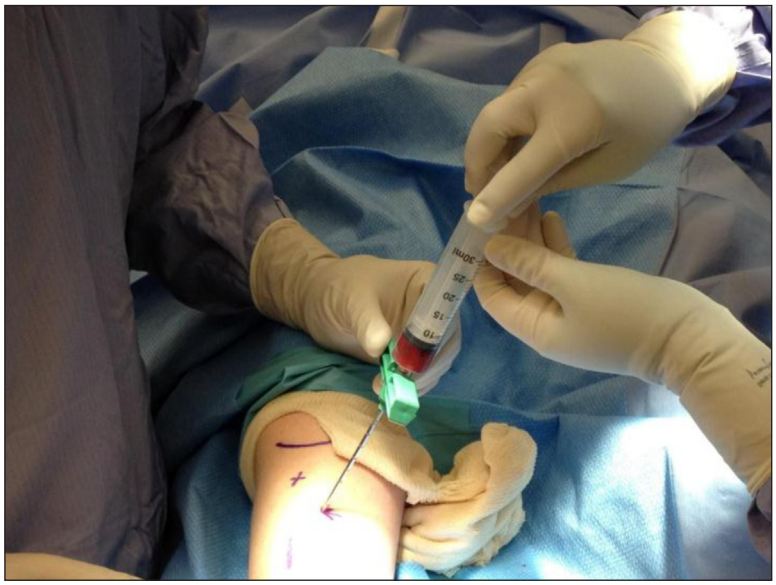


Figure 2: Obtainment of cBMA from tibia. Slow draw is key to avoid lysis of cells. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

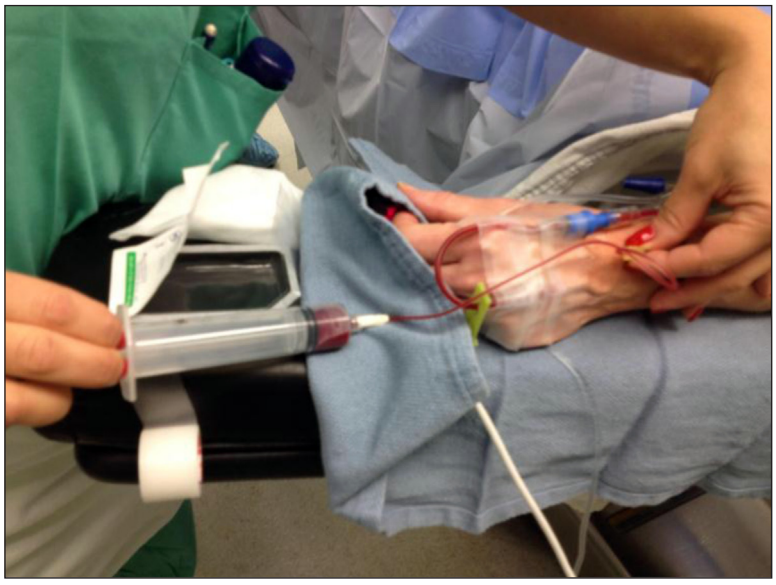


Figure 3: Obtaining peripheral blood for further cBMA. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

been shown to give rise to different mesodermal cell lineages including osteoblasts and chondroblasts [9]. This is critical to surgeons who are performing procedures involving bone lengthening for correction of deformities and arthrodesis interventions.

For bone lengthening procedures, fluoroscopic guided injection of the cBMA should occur in a stepwise fashion as the lengthening develops. The first occurrence happens at the time of the osteotomy, demonstrated in **Figure 5**. The purpose of this injection is to aid in the vascularization of the periosteum. This, in turn, will release the MSC to differentiate into osteoblasts to achieve bony growth [4]. After performance of osteotomy, gradual lengthening is occurred until desired goal is achieved. After lengthening, a second fluoroscopic guided injection of cBMA is used to assist in the remodeling and regeneration of the newly synthesized bone. This is done before the early consolidation phase, approximately 45 days after the initial osteotomy. Postoperative lengthening results are shown in **Figure 6**.

A problem with revisional surgeries that need arthrodesis is the loss of bone stock. Grafting with donor allograft is a viable option in these circumstances. Other grafting techniques have been presented to compensate for diminished bone stock, but present multiple limitations. Therefore, grafting with allograft currently appears to be one of the better choices. However, one of the problems with allograft is the lack of osteoprogenitor cells [3]. This is why the utilization of cBMA with the ability of the MSCs to differentiate is a vital part of the integration of the graft into the host tissue. The graft can be soaked into the cBMA before implantation, and then a fluoroscopic guided injection can be performed after closure to ensure that amount of progenitor cells remain high.

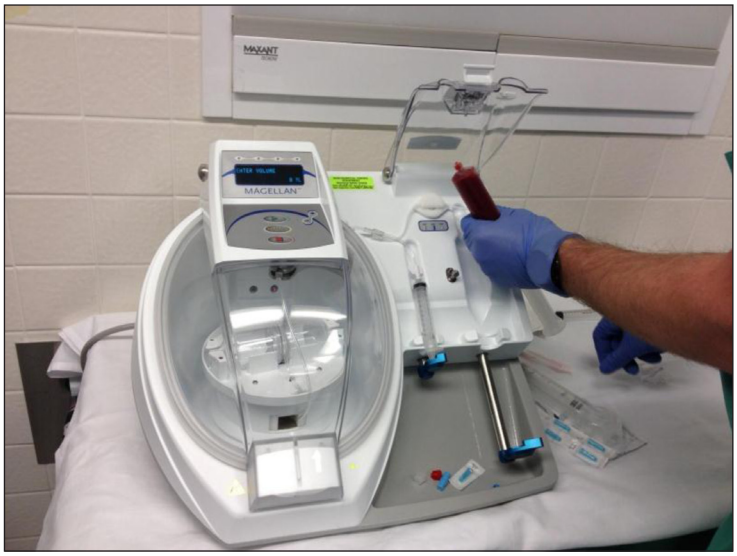


Figure 4: The Arteriocyte Magellan System, used to centrifuge the peripheral blood and bone marrow samples to obtain PRP and cBMA. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

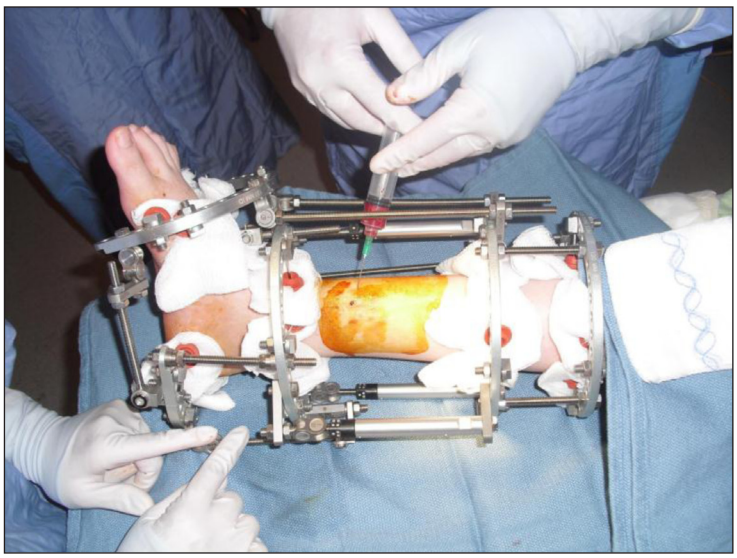


Figure 5: Percutaneous injection of cBMA into a tibial lengthening procedure. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

Summary for cBMA in Bone Surgery:

- cBMA has MSCs, which can differentiate into osteoblasts
- First cBMA injection occurs at first osteotomy to increase number of osteoblasts
- Second cBMA injection done when lengthening is complete to achieve remodeling/regeneration

Protocols for Pedicle Vascularized Muscular Transposition

One major factor contributing to chronic non-healing ulcerations is the lack of an adequate wound base in which proliferation of the wound can occur. Furthermore, disruption of the soft tissue envelope, either by surgical or non-surgical means, can leave little to no foundation to cover vital underlying structures [11]. One technique to attend to these wounds is the use of muscle flaps to cover and maintain a viable wound environment. This surgical technique is a salvage procedure to avoid need for limb amputation. Application of cBMA, with its rich source of MSCs and Hematopoietic Stem Cells (HSCs), offers the ability for increased regeneration and infusion of these flaps into the transposed site [7]. Furthermore, the implementation of Platelet Rich Plasma (PRP) releases various growth factors that are vital for the regeneration of a healthy wound base post muscle flap [11]. A logical speculation is that the bone marrow–derived stem cells and growth factors within the cBMA and PRP are useful in the prevention of muscle necrosis [10].

There are a multitude of approaches that can be employed when performing muscle flap surgery. One method is a staged approach. In this strategy, the muscle flap is elevated off of its soft tissue and bone attachments while preserving the blood supply that will be used post tissue transfer (**Figure 7**). At this time, the cBMA is injected around the muscular portion and into the muscle belly itself. This allows for the muscle flap to adjust to the new decrease

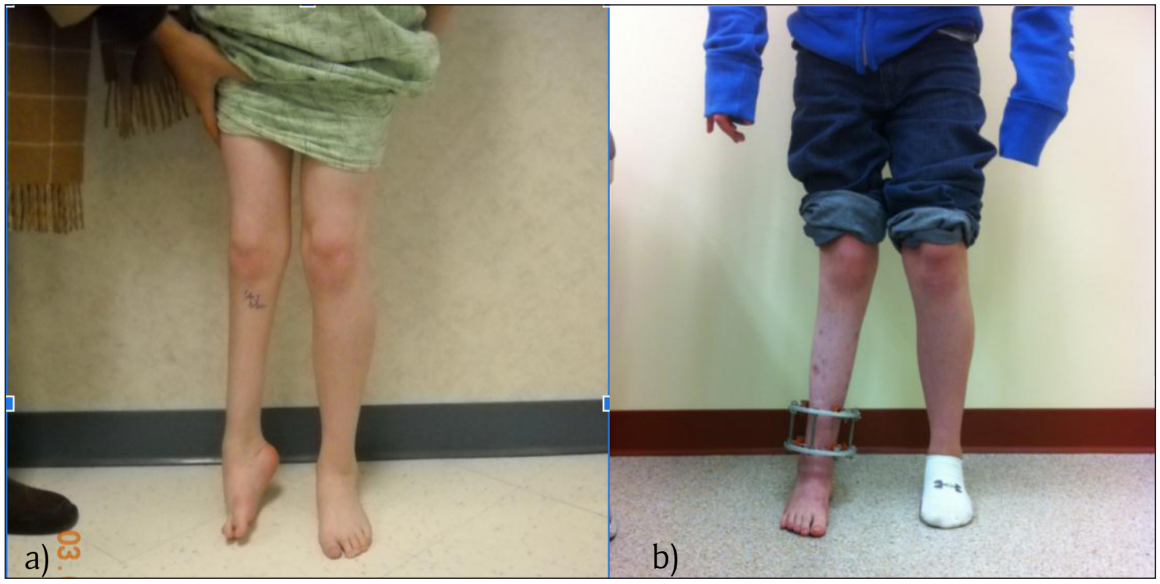


Figure 6: (a) Pre-limb deformity correction and (b) Post-correction and cBMA. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

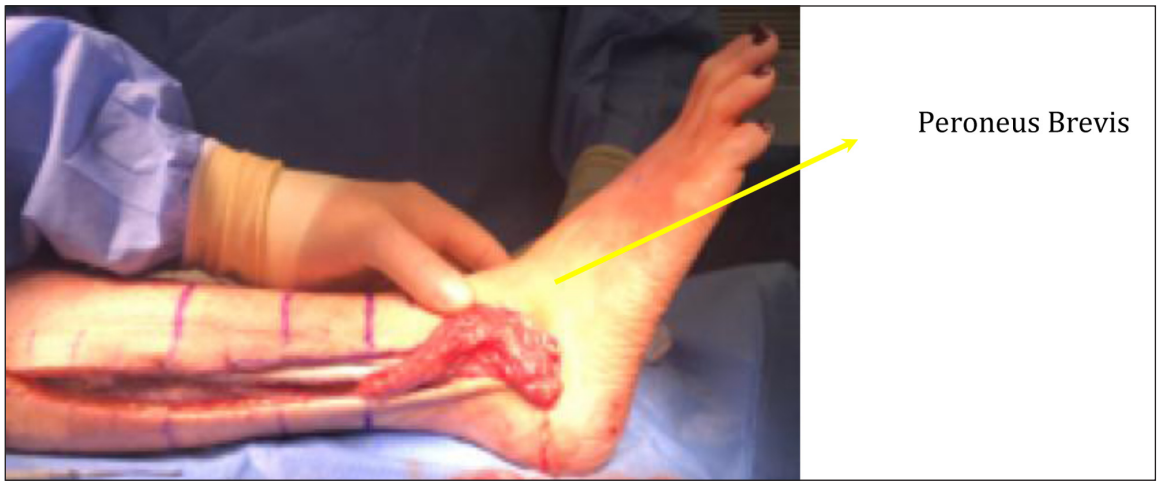


Figure 7: Elevation of Peroneus Brevis Muscle Flap. Photo: Stephen Frania. Reproduced with permission of the photographer.

in circulation with an increase in the neovascularization within the muscle itself, while preparing the muscle bed for transplant. Furthermore, this supplies the muscle belly with a rich amount of HSCs, decreasing the effects of the loss of blood supply. The next step would be to perform the transplantation, approximately 10 days after the initial elevation. A second cBMA injection would be performed, with injection at the recipient site as well as into the muscle belly and into the base of the muscle flap (**Figure 8**). The injection of the cBMA into the recipient site allows for the wound site to leave the inflammatory state and allows the MSCs to develop into healthy granular tissue, thus increasing the chance of acceptance of the muscle into the wound site.

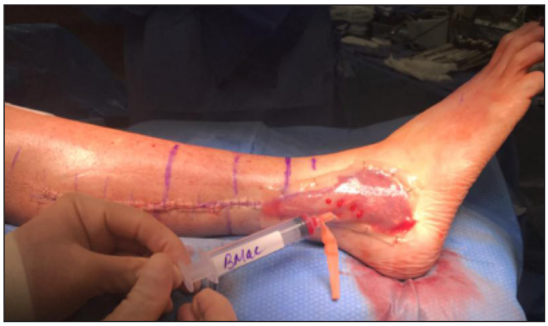


Figure 8: Injection of cBMA into elevated and transposed Peroneus Brevis flap with application of INTEGRA Bilayer Graft. Photo: Stephen Frania. Reproduced with permission of the photographer.

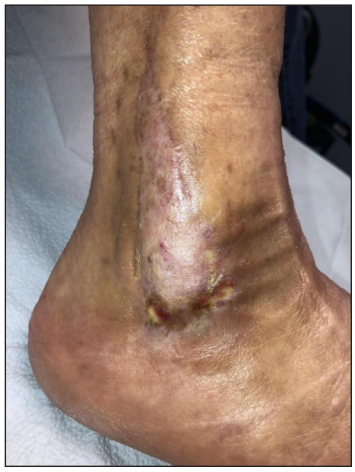


Figure 9: Postoperative result of muscle flap with cBMA. Photo: Timothy Miller. Reproduced with permission of the photographer.

Instead of a staging process as described above, the flap can be transferred in one stage with application of cBMA as described. Care is taken to leave as many perforator arteries to the muscle as possible in order to aid in adequate perfusion and decrease the risk of failure. Whichever method is used, the cBMA application is an important component to aid in the success of the transplant. After the muscle is moved, it is vital to begin the epithelialization process to further decrease the chance of failure. This process begins with the application of a regenerative dermal matrix, such as the INTEGRA Bilayer Mesh Graft (Integra LifeSciences Corp). This decreases the time it takes to grow a dermal matrix, and restores the form and function of the graft site [11]. Furthermore, this creates a vascularized layer to aid in application of a skin graft. The silastic layer of the graft aids in covering the wound to protect it from the outside environment as well. Application of Negative Pressure Wound Therapy (NPWT) at this time decreases the risk of hematoma/seroma, which increases the chance of take of the muscle [11].

After 7–10 days of continuous NPWT, the surgical site is checked. If the silicone layer of the INTEGRA is still adhered, the NPWT is continued for another 7 days until the silicone layer is off. Once the silicone layer is not adhered, an autologous split thickness skin graft is applied on top of the exposed muscle. At this time, PRP can be obtained and added to both the recipient and donor site. This will aid in hemostasis, and give an influx of growth factors to decrease the time of skin graft take and increase the rate of epithelialization [13]. Postoperative outcomes are shown in **Figure 9**.

Summary for cBMA in Muscle Transpositions:

- Two methods for muscle flaps
  - Staged: elevate muscle from bone/soft tissue, while preserving the blood supply that will be used after transfer. Inject cBMA into muscle belly to allow adjustment of decreased circulation. Second cBMA injection done after transplant (approximately 10 days after elevation), to allow do-

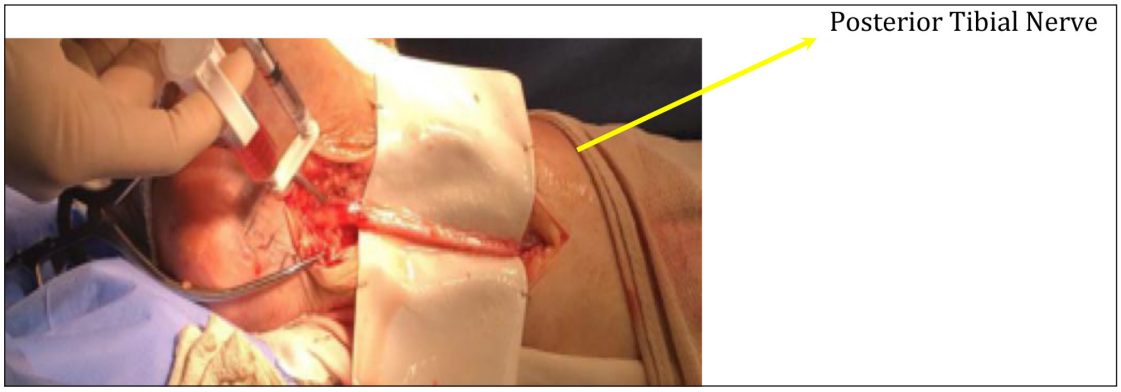


Figure 10: Injection of cBMA/PRP into nerve after neurolysis. Photo: Edgardo Rodriguez-Collazo. Reproduced with permission of the photographer.

nor site to accelerate in granulation tissue and aid in circulation to muscle flap
- One stage: cBMA done initially

Protocols for Micro Peripheral Nerve Reconstruction

Unlike other tissues in the body, peripheral nerve regeneration is slow and usually incomplete [6]. This presents a problem to surgeons who are looking to improve the lives of their patients affected with these conditions. Micro peripheral nerve reconstruction in the lower extremities is a viable option that can be offered to patients. cBMA can assist the surgical healing time and aid in the patient's recovery.

Direct nerve repair with epineural microsutures is still the gold standard surgical treatment for severe axonotmesis and neurotmesis injuries. Epineural repair is performed when a tension free co-optation in a well-vascularized bed can be achieved [6]. When there is a gap between the nerve ends with excessive tension for direct epineural repair, reversed interposition autologous nerve grafts are required [5]. Human cadaveric nerve allografts have been used in a limited number of patients with extensive nerve injuries and inadequate autologous nerve donor tissue.

Careful dissection is crucial when dealing with micro peripheral nerve reconstruction. Once the nerve in question is identified, cBMA can be used to improve the efficacy of the surgery. First, the concentrate can be applied after the dissection is complete (**Figure 10**). This not only controls bleeding intramuscularly, but it will inhibit the inflammatory response and decrease postoperative pain [9]. The use of cBMA is especially important when using an allograft or conduit assistive repairs. The MSCs and HSCs that are injected will assist in the neovascularization of the allograft/conduit, and promote nerve regeneration [12].

In patients experiencing foot drop, and a surgical transfer of the superficial peroneal nerve to the deep peroneal nerve is employed, the allograft that is used needs to be revascularized as soon as possible [1]. We believe that the cBMA helps to "force" the axons into the allograft, promoting regeneration. If revascularization is slow, a fibrous tissue will begin to form at the nerve ends, leading to a neuroma. In our experience this will lead to an ineffective surgery and a return of symptoms and preoperative pain for the patient.

Summary for cBMA in Micro Peripheral Nerve Reconstruction:

- cBMA used to control bleeding and decrease inflammation
- MSC and HSC assist in integration when graft is used. This allows for quicker revascularization which decreases the chance of fibrous formation

Conclusion

In recent years, intraoperative autologous cell therapy has emerged as an important and exciting approach that can potentially treat many medical conditions. Intraoperative approaches, employing different cell lineages, have already been shown to be safe and effective for multiple indications. Furthermore, many approaches are being developed to increase the therapeutic impact, optimize the desired outcome, and overcome current limitations. Cell therapy brings together a multitude of disciplines: biology, chemistry, biomaterials science, medicine, and engineering, among others [3]. Bone marrow aspirate concentration, along with platelet rich plasma, can be used to aid in an array of complex cases. PRP promotes antibacterial activity by releasing growth factors and cytokines [9]. One of the biggest factors of cBMA is the presence of the hematopoietic stem cells. With the addition of these cells, angiogenesis and neovascularization is possible and can aid in the acceptance of flaps/nerve repair [9]. This is especially important in immunocompromised patients. Presented here are the steps for obtaining bone marrow aspirate, along with the process of utilizing the concentrate in a multitude of surgical situations.

Funding Statement

There was no funding received for this paper.

Competing Interests

Dr. Rodriguez Collazo is a consultant for Isto Biologics. The authors have no financial interest to declare in relation to the content of this article.

Author Contributions

- Timothy J. Miller: wrote paper, performed procedures, provided pictures
- Edgardo Rodriguez-Collazo: wrote paper, performed procedures, provided pictures
- Stephen J. Frania: performed procedures, provided pictures
- Alessandro Thione: performed procedures

Guarantor

Edgardo Rodriguez-Collazo.

Peer Review

This is a non-commissioned paper that has undergone external peer review according to journal policy.

References

1. **Bibbo, C, Rodriguez-Collazo, ER** and **Finzen, A.** Superficial Peroneal Nerve to Deep Peroneal Nerve Transfer With Allograft Conduit for Neuroma in Continuity. *Journal of Foot and Ankle Surgery*. 2018; 57: 514–517. DOI: https://doi.org/10.1053/j.jfas.2017.11.022
2. **Çetinkaya, RA, Yenilmez, E, Petrone, P, Yilmaz, S, Bektore, B, Simsek, B, Kula Atik, T, Ozyurt, M** and **Unlu, A.** Platelet-rich plasma as an additional therapeutic option for infected wounds with multidrug resistant bacteria: in vitro antibacterial activity study. *European Journal of Trauma and Emergency Surgery*; 2018. DOI: https://doi.org/10.1007/s00068-018-0957-0
3. **Coelho, MB, Cabral, JM** and **Karp, JM.** Intraoperative stem cell therapy. *Annual Review in Biomedical*

Engineering. 2012; 14: 325–349. DOI: https://doi.org/10.1146/annurev-bioeng-071811-150041

4. **Gianakos, AL, Sun, L, Patel, JN, Adams, DM** and **Liporace, FA.** Clinical application of concentrated bone marrow aspirate in orthopaedics: A systematic review. *World Journal of Orthopedics*. 2017; 8(6): 491–506. DOI: https://doi.org/10.5312/wjo.v8.i6.491
5. **Griffin, MF, Malahias, M, Hindocha, S** and **Khan, WS.** Peripheral nerve injury: principles for repair and regeneration. *Open Orthopedics Journal*. 2014; 8: 199–203. DOI: https://doi.org/10.2174/1874325001408010199
6. **Grinsell, D** and **Keating, CP.** Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. *Biomedical Research International*; 2014. DOI: https://doi.org/10.1155/2014/698256
7. **Hauser, RA** and **Orlofsky, A.** Regenerative injection therapy with whole bone marrow aspirate for degenerative joint disease: a case series. *Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders*. 2013; 6: 65–72. DOI: https://doi.org/10.4137/CMAMD.S10951
8. **Mishra, A, Tummala, P, King, A, Lee, B, Kraus, M, Tse, V** and **Jacobs, CR.** Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. *Tissue Engineering Part C: Methods*. 2009; 15(3). DOI: https://doi.org/10.1089/ten.tec.2008.0534
9. **Rodriguez-Collazo, ER** and **Maria, ML.** Combined use of the Ilizarov method, concentrated bone marrow aspirate (cBMA), and platelet-rich plasma (PRP) to expedite healing of bimalleolar fractures. *Strategies in Trauma and Limb Reconstruction*. 2015; 10(3): 161–166. DOI: https://doi.org/10.1007/s11751-015-0239-x
10. **Rodriguez-Collazo, ER, Rathbone, CR** and **Barnes, BR.** A Retrospective Look at Integrating a Novel Regenerative Medicine Approach in Plastic Limb Reconstruction. *Plastic Reconstructive Surgery Global Open*. 2017; 5(1): e1214. DOI: https://doi.org/10.1097/GOX.0000000000001214
11. **Rodriguez-Collazo, ER, Weiland, J, Manson, TS** and **Jones, A.** Peroneus Brevis Muscle Flap with the use of INTEGRA® Wound Matrix and Split Thickness Skin Graft in the Treatment of Full Thickness Ulcerations: Case Reports and Technique Guide. *Orthopedics and Rheumatology Open Access Journal*. 2015; 1. DOI: https://doi.org/10.19080/OROAJ.2015.01.555567
12. **Sayad Fathi, S** and **Zaminy, A.** Stem cell therapy for nerve injury. *World Journal of Stem Cells*. 2017; 9(9): 144–151. DOI: https://doi.org/10.4252/wjsc.v9.i9.144
13. **Vural, E, Berbée, M, Acott, A, Blagg, R, Fan, CY** and **Hauer-Jensen, M.** Skin graft take rates, granulation, and epithelialization: dependence on myeloid cell hypoxia-inducible factor 1alpha. *Archives Otolaryngology Head & Neck Surgery*. 2010; 136(7): 720–723. DOI: https://doi.org/10.1001/archoto.2010.103

How to cite this article: Miller, TJ, Rodriguez-Collazo, E, Frania, SJ and Thione, A. Regenerative Surgery & Intra-Operative Protocols Utilizing Bone Marrow Aspirate Concentrate in Microsurgical & Limb Reconstruction. *International Journal of Orthoplastic Surgery*. 2019; 2(2), pp. 39–46. DOI: https://doi.org/10.29337/ijops.29

Submitted: 24 December 2018 **Accepted:** 26 March 2019 **Published:** 26 April 2019

International Journal of Orthoplastic Surgery is a peer-reviewed open access journal published by IJS Publishing Group.

OPEN ACCESS