Stem Cells in Bone Grafting: Trinity Allograft with Stem Cells and Collagen/ Beta-Tricalcium Phosphate with Concentrated Bone Marrow Aspirate

Gregory P. Guyton, MD, Stuart D. Miller, MD*

KEYWORDS

• Bone graft • Trinity allograft • Beta-tricalcium phosphate
• Mesenchymal stem cells

The orthopedic foot and ankle surgeon needs bone grafts in the clinical situation of fracture healing and in bone-fusion procedures. Although the need for graft remains controversial for some procedures such as triple arthrodesis, many surgeons prefer to augment bone-on-bone healing with biology as well as to fill the gaps or "dead space" left after some procedures. The need for some sort of graft with traumatic cavitation or fracture comminution warrants even further merit. Frank nonunions or resection of bone, such as for avascular necrosis, infection, or tumor, provide uncontroversial need for bone graft filling. This article briefly outlines thought processes and techniques for 2 recent options for the surgeon. The Trinity product is a unique combination of allograft bone and allograft stem cells. The beta-tricalcium phosphate and collagen materials provide an excellent scaffold for bone growth; when combined with concentrated bone marrow aspirate they also offer osteoconductive and osteoinductive as well as osteogenerative sources for new bone formation.

Department of Orthopaedic Surgery, Union Memorial Hospital, 3333 North Calvert Street, Suite 400, Baltimore, MD 21218, USA
* Corresponding author.
E-mail address: smiller@gcoa.net

Foot Ankle Clin N Am 15 (2010) 611–619
doi:10.1016/j.fcl.2010.09.003 **foot.theclinics.com**
1083-7515/10/$ – see front matter

PART I. TRINITY EVOLUTION AS AN ALLOGRAFT AUGMENTATION

Trinity Evolution (Orthofix International NV, Boston, MA, USA) is a proprietary allograft formulation specifically created to contain 3 separate elements to induce bone healing. These include:

1. Living osteogenic cells (both mature osteoblasts and osteoprogenitor cells)
2. An osteoconductive matrix
3. Osteoinductive cytokines.

The material is essentially a mixture of a consistent and very high concentration of mesenchymal stem cells, a fine cancellous bone matrix, and an admixture of demineralized cortical bone. In concept, osteogenesis, osteoconductivity, and osteoinduction are supplied by the 3 components respectively.[1]

GRAFT HANDLING

The graft itself is processed sterilely from the donor and the mesenchymal stem cell component is concentrated by a proprietary process. It can be stored on site after shipping at –70°C to –80°C for up to 3 months and must be used immediately once thawed. Thawing is accomplished in the operating room at a temperature no greater than 39°C to avoid cellular necrosis. A fluid component is present in the preparation including dimethyl sulfoxide (DMSO) cryoprotectant and the mesenchymal stem cell–specific basal medium. This is decanted after thawing immediately before implantation (**Fig. 1**). The thawing protocol must be followed precisely, as loss of stem cell viability may otherwise result.

MESENCHYMAL STEM CELLS IN HUMAN USE

Mesenchymal stem cells (MSCs) are a population of adult mesenchymal cells that can initiate a differentiation pathway into multiple different connective and bony tissues. Multiple roles for MSCs in vivo have been described, including serving as progenitor cells for bone remodeling and repair,[2] cartilage formation,[3] vascular support,[4] hematopoietic support,[5] and as progenitors for adipocytes.[3] MSCs have been proposed for a role in a variety of human tissue engineering applications, including the treatment of nonunions or supporting healing in high-risk fusion procedures.

Recent data suggest that MSCs naturally occur as perivascular cells (formerly called “pericytes”) that are released at zones of injury. Activated MSCs then secrete large amounts of trophic and immunomodulatory cytokines. The trophic characteristics stimulate the tissue angiogenesis critical for healing as well as simulator local tissue progenitor cells. The resultant healing tissue, then, is primarily the result of the activation of the healing process of the surrounding tissue rather than directly derived from the MSCs themselves.[6]

MSCS AS AN ALLOGRAFT

The immunomodulatory cytokines are particularly important. They inhibit host lymphocyte surveillance of the injured tissues and prevent a large-scale autoimmune response.[1] The immunomodulatory characteristics of MSCs allows for the use of cells of allogeneic origin. Even the use of xenograft-sourced MSCs has been explored. Culture-expanded MSCs do not appear to elicit a significant host immune response even when directly infused intravenously.[7]

Fig. 1. Trinity is stored in a plastic vial at –70 to –80°C. It is slowly thawed to no more than 39°C in the operating room at the time of use. The material has the handling characteristics of soft cancellous bone.

MSCs have been demonstrated to show remarkable potential for healing of defects in long bones in multiple animal studies, and their use as an allograft material with intraoperative bone marrow concentration has gained increasing attention. As a practical matter, surgical use of MSCs is restrained by both the concern that without a carrier the grafted cells will not remain in the desired location and the variable quality of MSCs generated from the patient's own tissue. In particular, increasing patient age significantly affects the concentration of MSCs that can be generated from autograft concentration techniques. There is also increasing evidence that the relative "fitness" of MSCs may be reduced with age. Stolzing and colleagues[6] recently demonstrated multiple indices of aging including oxidative damage, reactive oxygen species (ROS) levels, p21, and p53 markers all increasing in older patient harvests. Other host factors, such as smoking and diabetes, also play a role. Unfortunately, it is these same factors that often place the patient into a high-risk category requiring a graft for augmentation in the first place.

THE RATIONALE BEHIND TRINITY

Trinity seeks to avoid these issues. First, the MSCs are adherent to the extracellular matrix of the cancellous material, thus providing a human allograft carrier to help ensure the cells remain in place. Second, efforts are made to take the material from younger donors; the average donor age for harvests in 2008 was 30. Assay of each preparation ensures a minimum concentration of 50,000 mesenchymal stem cells or osteoprogenitor cells per mL.[8]

SUITABLE USAGE

The appropriate indications for the use of Trinity Evolution, like other augmentation allograft products in foot and ankle surgery, remain ill defined. Classically autograft supplementation is considered in cases of substantial nonunion gaps, smokers, steroid users, and patients with chronic diseases such as diabetes. Although all methods may be said to have advantages and disadvantages, the distinction between Trinity and autograft MSC concentration techniques is most clear in the older or high-risk patient groups where native MSC concentration and activity may be suspect. In addition, the concentrations of MSCs achievable through culture expansion and concentration are substantially higher than through intraoperative centrifuge bone marrow concentration. Whether or not these theoretical advantages supply actual clinical benefit awaits carefully designed clinical trials.

PART II: BETA-TRICALCIUM PHOSPHATE/COLLAGEN WITH CONCENTRATED BONE MARROW ASPIRATE

When making a decision for bone grafting, the surgeon and patient may be unwilling to harvest bone from a second site. A bone graft substitute, such as beta-tricalcium phosphate with collagen, offers excellent biologic scaffold and dead space filler. The augmentation of this bone substitute with concentrated bone marrow aspirate potentially offers an even more attractive bone graft option.

The science of beta-tricalcium phosphate as a bone graft material dates far back in orthopedic history, when calcium phosphate (plaster of Paris) was found to be a resorbable bone graft substitute. Although various combinations of calcium and sodium phosphates have been tried, the microstructure of beta-tricalcium phosphate seems to be very close to cancellous bone. The incorporation of collagen into the beta-tricalcium phosphate binds proteins such as those within bone marrow aspirate. The collagen binds the proteins via electrostatic attraction forces.[9] Two commercial varieties of beta-tricalcium phosphate with collagen, IntegraOS (IntegraLifesciences, Inc, Plainsboro, NJ, USA) and Vitoss (Orthovita, Inc, Malvern, PA, USA), may differ in microstructure but clinically handle almost identically. Supplied in a 5-mL-long rectangular block, the materials resemble a tough Styrofoam and can be broken or cut into smaller pieces to fit small holes or gaps. Several varieties of beta tri-calcium phosphate have been produced, and definitive clinical outcome articles have yet to discern the best-performing graft in vivo. Although these materials are approved by the Food and Drug Administration (FDA) as bone graft, both manufacturers promote the use along with concentrated bone marrow aspirate.[10,11] Orthovita has also produced a next-generation Vitoss BA, which has added bioglass in an attempt to speed bone incorporation. The silicon and sodium liberated by the bioglass in theory attracts osteoblasts and thus speeds bone formation.[12]

The use of bone marrow aspirate (BMA) has markedly increased over the past several years in our institution as more information regarding the composition of the BMA has been found. The stem cells compose the primary element sought for differentiation into bone-forming cells and the iliac crest has a higher concentration of mesenchymal stem cells than the proximal tibia or the heel (Lew C. Schon, internal data at Union Memorial Hospital Orthobiologics Laboratory, 2009). Interestingly, the proximal tibia had the lowest concentration of stem cells, a worrisome issue for those still harvesting bone graft from that location. The other components of the concentrated bone marrow include a myriad of growth factors and cytokines; the qualitative and quantitative aspects of each are still being discovered. Still to be published are comparison studies of concentrated BMA (with or without carrier) to MSC

preparations (such as Trinity). These MSC preparations, mixed with allograft cancellous bone, present a very attractive bone graft alternative to autograft in some patients.

SURGICAL TECHNIQUE FOR BONE MARROW ASPIRATION

The process of BMA has evolved into a simple procedure. Preoperatively, we usually wall off the pelvic area from the anterior iliac crest with an unsterile U-drape, then prep the crest at the same time as the leg and foot. We will usually block the anterior iliac crest with 5 to 10 mL of a lidocaine/bupivicaine solution, first the skin then the deep periosteum of the anterior iliac crest. In addition to pain control, this procedure helps confirm the location of the crest. The bordering of the crest with sterile towels is optional. We do take care not to drag the large drape over unprepped areas of the thigh or hip as we lay down the single-holed sterile drape from the leg cephalad toward the head. We cut a hole for the crest and use a clear adhesive drape over this hole to secure the crest for aspiration.

The aspiration begins with a simple incision using half of the #15 blade into the skin over the anterior iliac crest, a few centimeters behind the anterior superior iliac spine (ASIS). This small stab wound makes a much more cosmetic scar than the hole from the BMA trocar. The Biomet GPS (Biomet, Inc, Warsaw, IN, USA) harvesting trocar is then percutaneously advanced to the iliac crest and the width of the bone can be felt. The central aspect of the crest can then be entered by using a mallet to advance the trocar between the walls of the crest (**Fig. 2**). Once within, the simple aspiration into a 30-mL syringe, prefilled with 4 mL of ACD-A (acid citrate dextrose-anticoagulant),

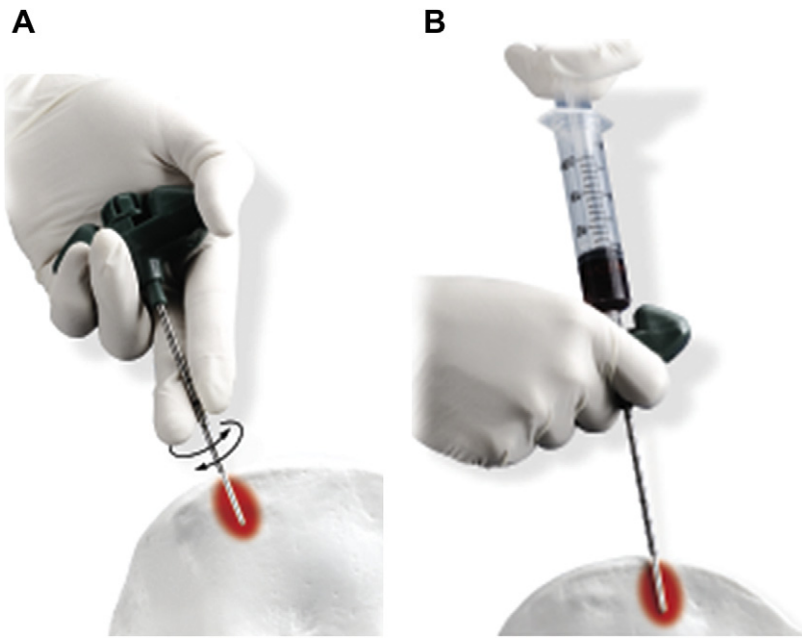

Fig. 2. (*A*) Use of trocar to harvest bone marrow between iliac walls. A mallet can also be used to advance the trocar. (*B*) Withdrawing a small amount from each segment of crest, trying to maximize bone marrow and minimize peripheral blood. (*Courtesy of* Biomet, Inc; with permission.)

yields the BMA. The choice of 30 or 60 mL of BMA depends on the volume of graft needed as well as the age and physiology of the patient. Younger, healthy patients can be expected to yield more BMA than older osteoporotic patients. Harvesting technique can vary greatly, as too much fluid aspiration at one site will begin to mix peripheral blood with bone marrow; we try to limit harvest to 10 mL from each trocar plunge.

Once collected, the BMA gets passed off to a technician who spins it down in a centrifuge with proprietary filters (Biomet GPS). This spin-down process yields 2 batches of BMA concentrate, labeled from the previous generation of peripheral blood spin-downs, PRP (platelet-rich plasma) and PPP (platelet poor plasma) (**Fig. 3**). The PRP represents the marrow stem cell–rich portion of the BMA. The block of beta-tricalcium phosphate /collagen can then be broken into several small 1- to 2-mL blocks and soaked in the PRP stem cell–rich aspirate. The smaller sizes allow for better and more rapid absorption of the aspirate into the graft. This absorption may take several minutes and thus should be performed well ahead of the timing for graft application.

The bone graft can be applied once the PRP is well absorbed; the material handles easily and compacts well into small crevices and spaces (**Figs. 4** and **5**). It has minimal strength in compression or tension and can be used to augment a corticocancellous graft (we like to burr a central groove or pack along the outside of such a graft) or cancellous chips. The product brochure for Mozaic (a different configuration of the beta-tricalcium phosphate and collagen from IntegraLifesciences) states that this configuration has much better compression resistance but these authors' experience is limited. The surgeon should take care to irrigate the surgical site before placement of the graft because the stem cell–rich iliac crest bone marrow aspirate–beta-tricalcium phosphate graft should not be diluted with saline after instillation. The graft can be mixed with allograft; some have even augmented the graft with bone morphogenic protein-2 in complex cases to promote osteogenesis. Another promotion of bone growth can be an internal or external bone stimulator, although studies of the benefits

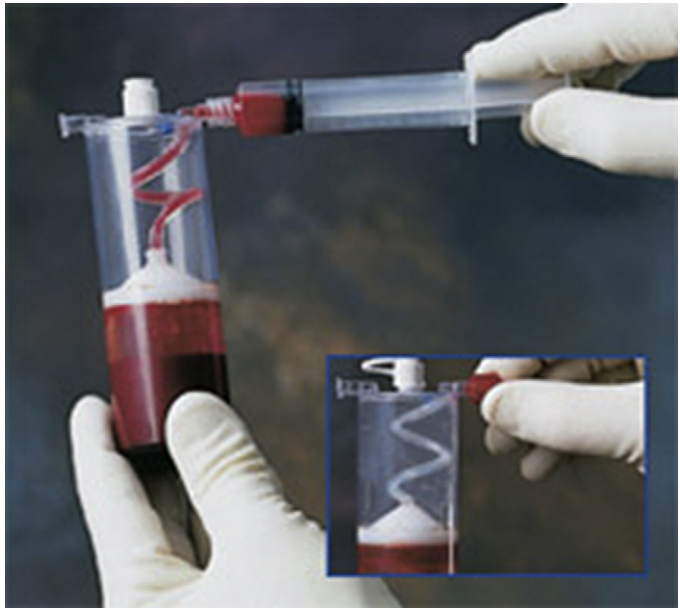

Fig. 3. Separation of PRP and PPP. (*Courtesy of* Biomet, Inc; with permission.)

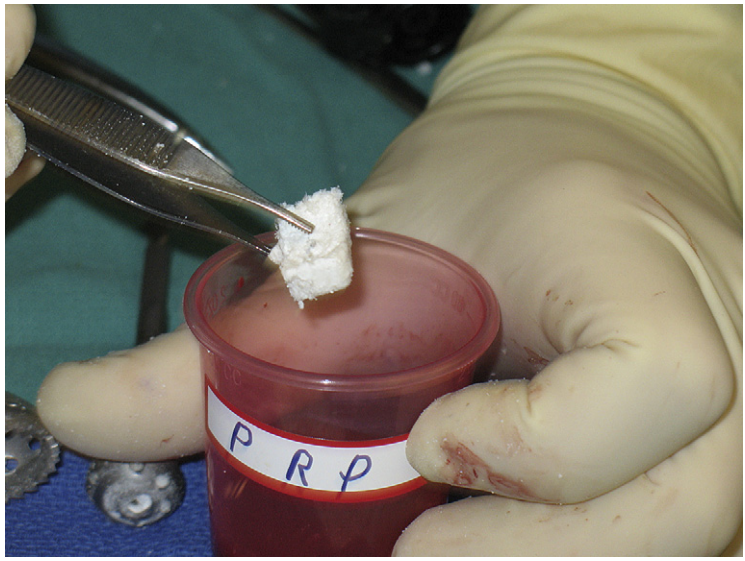

Fig. 4. Tricalcium phosphate/collagen material broken into a smaller piece.

with a bone marrow/beta-tricalcium phosphate graft have not been performed to these authors' knowledge.

The PPP component of the bone marrow aspirate has garnished an anecdotal reputation for great utility in wound healing. Several surgeons use the fluid in the subcutaneous tissues to diminish inflammation; the PPP is injected in the subcutaneous tissue after wound closure. Although no hard data have been presented, these authors have also enjoyed beneficial results over several years using this technique, described by fellow surgeon Lew C. Schon (personal communication, 2008).

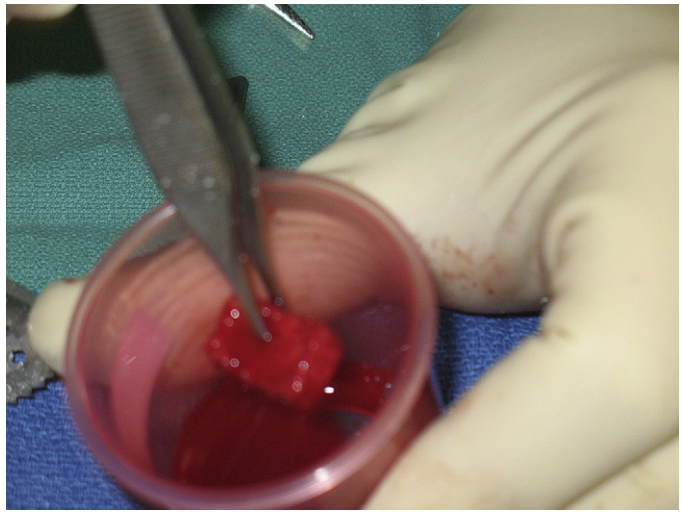

Fig. 5. After soaking IntegraOS in the concentrated bone marrow aspirate.

SUMMARY

The concept of osteoinduction and osteoconduction has been furthered with the concept of osteogenesis; Siegel and colleagues[13] discuss the scaffolding of the tricalcium phosphate/collagen for osteoconduction, the growth factors in the concentrated bone marrow aspirate for osteoinduction, and the stem cells in the concentrated bone marrow aspirate as further stimulus for osteogenesis. The scientific studies have led to various conclusions regarding the best combination of substrate and biologic agent. Castellani and colleagues[14] found no improvement in bone ingrowth between TricOs (Baxter AG, Vienna, Austria) and Collagraft (Zimmer, Inc, Warsaw, IN, USA), both tricalcium phosphates with a bioactive matrix, when bone marrow aspirate was added to the ceramics filling a rabbit femoral defect. In contrast, Liu and colleagues[15] found that human bone marrow stromal cells greatly augmented the bone formation in beta-tricalcium phosphate scaffolds when implanted into mice. Hing and colleagues[16] discussed finding in spine grafting that some of the tricalcium phosphates might be degraded early, thus decoupling bone regeneration and resorption and leading to incomplete bone repair. Their studies led to conclusion that a silicone-calcium phosphate led to a more stable osteoconductive scaffold, which thus better supported angiogenesis and bone apposition.

Recent spine work has confirmed clinical success of the beta-tricalcium phosphate/collagen combination. The beta-tricalcium phosphate has been used clinically with allograft[17] and autograft[18,19] for lumbar interbody fusion. The beta-tricalcium phosphate has been compared clinically with iliac crest autograft for posterior correction of scoliosis with equivalent fusion and less morbidity.[20] This entire issue could be devoted to comparing and contrasting the myriad of studies available; a consensus has yet to be clearly made as to the best combination of materials for bone grafting and augmentation. The authors have found great clinical success using the material in the foot and ankle, especially with concentrated bone marrow aspirate, and clinical outcome studies are under way.

REFERENCES

1. Ryan JM, Barry FP, Murphy JM, et al. Mesenchymal stem cells avoid allogeneic rejection. J Inflamm (Lond) 2005;2:8.
2. Blair HC, Zaidi M, Schlesinger PH. Mechanisms balancing skeletal matrix synthesis and degradation. Biochem J 2002;364:329–41.
3. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
4. Hegner B, Weber M, Dragun D, et al. Differential regulation of smooth muscle markers in human bone marrow-derived mesenchymal stem cells. J Hypertens 2005;23:1191–202.
5. Jang YK, Jung DH, Jung MH, et al. Mesenchymal stem cells feeder layer from human umbilical cord blood for ex vivo expanded growth and proliferation of hematopoietic progenitor cells. Ann Hematol 2006;85:212–25.
6. Stolzing A, Jones E, McGonagle D, et al. Age-related changes in human bone marrow-derived mesenchymal stem cells: consequences for cell therapies. Mech Ageing Dev 2008;129:163–73.
7. Caplan A. Why are MSC's therapeutic? new data: new insight. J Pathol 2009;217:318–24.
8. Musculoskeletal transplant foundation, Orthofix, Verona, Italy. "Trinity evolution" clinical data sheet 2009.
9. Mozaic product brochure. Integra life sciences, Inc, Plainsboro (NJ); 2008.

10. Vitoss product brochure. Orthovita Inc, Malvern (PA); 2009.
11. Ullrich PF. Interbody fusion using VITOSS scaffold with autogenous bone marrow aspirate—one year interim results. Orthovita, Malvern (PA): Internal White Paper Publication; 2006.
12. The use of bioactive glass in orthopaedics: improvements in healing with vitoss bioactive glass substitute, a white paper from Orthovita, Inc, Malvern (PA); 2008.
13. Siegel HJ, Baird RC, Hall J, et al. The outcome of composite bone graft substitute to treat cavitary bone defects. Orthopedics 2008;31(8):754.
14. Castellani C, Zanoni G, Tangl S, et al. Biphasic calcium phosphate ceramics in small bone defects: potential influence of carrier substances and bone marrow on bone regeneration. Clin Oral Implants Res 2009;20(12):1367–74.
15. Liu G, Zhao L, Cui L, et al. "Tissue engineered bone formation using human bone marrow stromal cells and novel beta-tricalcium phosphate". Biomed Mater. 2007; 2(2):78–86.
16. Hing KA, Wilson LF, Buckland T. Comparative performance of three ceramic bone graft substitutes. Spine J 2007;7(4):475–90.
17. Linovitz RJ, Peppers TA. Use of an advanced formulation of beta-tricalcium phosphate as a bone extender in interbody lumbar fusion. Orthopedics 2002; 25(5):S585–9.
18. Meadows GR. Adjunctive use of ultraporous beta-tricalcium phosphate void filler in spinal arthrodesis. Orthopedics 2002;25(5):s579–84.
19. Epstein NE. Non-instrumented posterolateral lumbar fusions utilizing combined lamina autograft and beta tricalcium phosphate in a predominately geriatric population: an outcome assessment. Spinal Surg 2006;20(4):219–31.
20. Lerner T, Bullmann V, Schulte TL, et al. A level-1 pilot study to evaluate ultraporous beta-tricalcium phosphate as a graft extender in the posterior correction of adolescent idiopathic scoliosis. Eur Spine J 2009;18(2):170–9.