The Role of Intraoperative Bone Marrow Aspirate Stem Cell Concentration as a Bone Grafting Technique

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ABSTRACT

Despite the recapitulative nature of bone, a large percentage of nonunions still occur. Many exogenous substances have been administered to aid in the arthrodesis process, yet few have proven efficacious relative to time and cost. With recent advancements in bone biology involving cellular and molecular signaling, it is now possible to selectively retain bone progenitor cells from a patient’s bone marrow. The commercially available product, Cellect™ Cellect selective cell retention device has gained attention for its fast and cost-effective intraoperative concentration of these bone progenitor cells from bone marrow aspirate. Application of this concentrate within a bone graft matrix of either tricalcium phosphate granules or demineralized bone matrix is a novel grafting technique that facilitates bony bridging of osseous defects.

Keywords: Cellect, selective cell retention (SCR), nonunions, graft matrix, mesenchymal stem cell

HISTORICAL PERSPECTIVE

Bone is a unique tissue due to its ability to both maintain regulatory metabolic turnover and regenerate due to injury. However, despite the recapitulative nature of bone, an increase of 46% of nonunions still may occur after long-bone fracture. Although a variety of treatments are available to aid in osseous bridging due to these defects, few implants or techniques have been completely successful.

Several approaches have been used to elicit bone formation and promote bone healing. The standard technique used to facilitate the arthrodesis process is the application of autogenous bone graft. Iliac crest graft harvesting remains today’s gold standard because autogenous graft is currently the only material that contains the 3 essential bone formation elements. However, autogenous bone graft (ABG) comes with significant costs.

Harvest is associated with significant clinical morbidity in terms of pain, scarring, increased surgical time, prolonged hospitalization, delayed rehabilitation, increased blood loss, increased risk of infection, and surgical complications (fracture, hematoma, neuroma, etc). A review of the literature reveals that complications arise in as many as 31% of the procedures, and 27% of patients undergoing ABG continue to feel pain at 24 months after surgery. Often the quantity of available graft may be less than optimal. For these reasons, an impetus exists to develop and validate alternative graft processes that are capable of replicating the performance of the iliac crest graft while eliminating the associated complications.

Due to the rapidly advancing knowledge of bone biology, it is now possible to exploit the potential advantages that cellular and molecular expression information has to offer. The cellular events that allow bone healing to occur include chemoattraction, migration, proliferation, and differentiation. The mesenchymal stem cell (MSC) population is the fundamental ancestor of this assemblage. Bone marrow is a milieu containing these unique progenitor cells that possess the ability to differentiate into bone, cartilage, tendon, and other connective tissues. Using aspirative techniques with the aid of cell retention technology and an osteoconductive scaffold, surgeons are able to selectively choose bone progenitor cells and use their differentiation capacity in the presence of a nonunion for better bone healing.

Several experimental approaches have been used to elicit the formation of bone within defects and to promote
their healing.\textsuperscript{4–6} Methodology used in such studies has been based on a strategy of ex vivo expansion of pluripotent MSCs that are loaded into a carrier system. This approach is based on the hypothesis that such a technique decreases the need for osteoblast progenitor cell chemotaxis into the defect as well as need for massive proliferation. The potential benefits of direct osteogenic progenitor cell implantation lie within its potential to produce more rapid, uniform, and reliable bone defect healing.

A review of the literature of ex vivo expansion of autologous and allogenic stem cell augmentation has demonstrated favorable results. Bruder et al analyzed autologous MSCs isolated from bone marrow, grown in culture and loaded into porous ceramic cylinders consisting of hydroxyapatite (65\%) and β-tricalcium phosphate (TCP) ceramic (35\%) in canine segmental bone defect model. The results demonstrated significant effect of the stem cell augmentation. At 16 weeks, the atrophic nonunion occurred in all of the femora that had untreated defect, with small amount of trabecular bone at the cut ends. In contrast, radiographic union was established rapidly at the interface between the host bone and the implants loaded with MSCs. In the percentage of histological and morphometric analysis, which demonstrated that both woven and lamellar bone had filled the pores of the implants that had been loaded with MSCs, the amount of bone was significantly greater ($P < 0.05$) than that found in the pores of the implants that had not been loaded with cells.\textsuperscript{3}

Arinzeh et al analyzed the application of allogenic MSCs in a critical-sized canine segmental defect. For defects treated with allogenic MSC implants, no adverse host response could be detected at any time point.\textsuperscript{5}

Histologically, by 8 weeks, a callus spanned the length of the defect and lamellar bone filled the pores of the implant at the host bone-implant interface. At 16 weeks, new bone had formed throughout the implant. The results were consistent with those seen in implants loaded with autologous cells. Implants loaded with allogenic or autologous stem cells had significantly greater amounts of bone within the available pore space than did cell-free implants at 16 weeks ($P < 0.05$).\textsuperscript{5}

Although the concept of ex vivo expansion of MSCs has demonstrated its potential clinical role in surgical arthrodesis, concerns regarding sterility issues and logistics of ex vivo expansion have led to lack of clinical acceptance.

The Cellect selective cell retention (SCR) system is a recently developed alternative to ex vivo expansion of MSCs for use in arthrodesis procedures. This intraoperative Cellect\textsuperscript{TM} process uses a pioneering selective retention technology that consists of osteogenic progenitor cell concentration/augmentation, achieving a 3- to 4-fold increase in osteoprogenitor cell concentration using a minimally invasive approach at the time of surgery. Mesenchymal stem cells possess the unique ability to differentiate into a number of phenotypes. The stem cell activity and differentiation is regulated by a series of naturally occurring growth factors that guide them to form bone-producing cells.\textsuperscript{5} The bone marrow is an ideal source of stem cells containing 2 primary stem cell lineages: hematopoietic and mesenchymal, the latter of which contains the potential to differentiate into the osteoprogenitor cell. Analysis of the bone marrow aspiration reveals that 50 million cells may be obtained in 2 mL of aspiration, of which only 1 per 20,000 cells being part of the osteoprogenitor lineage.\textsuperscript{9} Therefore, the strategy behind this selective retention technology provides a rapid, safe, simple, and inexpensive method for intraoperative concentration and delivery of bone marrow-derived cells that may improve the outcome of bone grafting.

Few studies exist regarding the effect of MSC augmentation on bone repair. Kadiyala et al presented data regarding the application of bone graft prepared with SCR technology in canine defect model. Using the process described above, 4 different bone graft materials were evaluated: autologous bone, enriched SCR allograft matrix, allograft matrix saturated with whole bone marrow, and allogenic matrix alone. At 4 weeks, all samples of the enriched SCR matrix (100\%) and autograft (100\%) groups demonstrated early evidence of cortical bridging. At 16 weeks, only the enriched SCR matrix group and autograft group showed complete cortical bridging by radiograph and micro-computed tomography. In contrast, sites grafted with the allograft matrix alone (50\%) or with bone marrow saturation (67\%) consistently lagged in healing progression. This study exhibits the potential role of this intraoperative SCR process.\textsuperscript{6}

An intraoperative iliac crest aspirate may be taken from a patient using an anterior or posterior approach. Alternatively, marrow may be aspirated from other locations such as the distal tibia or calcaneus. Allograft size requirements of varying volumes are easily accommodated because the bone marrow–seeded graft material is easily separated after being run through the selective retention device. Once the aspirate is isolated, prehydrated Conduit TCP granules or LifeNet CEL25 demineralized bone matrix (DBM) is loaded into the Cellect selective retention device. The marrow aspirate, in conjunction with heparin, is then added to this prehydrated matrix using a uniquely developed cartridge that aids in the selection of osteoblastic progenitor cells. The Conduit TCP granules are a synthetic porous ceramic graft material that closely mimics the mineral phase that comprises 70\% bone. Conduit TCP is a scaffold that allows for neovascularization and is resorbed as normal bone healing progresses. The LifeNet CEL25 model uses a DBM cortical fiber/cancellous chip matrix.\textsuperscript{10} Also used in
conjunction with heparin, this model incorporates DBM’s osteoinductive and osteoconductive effects on growth factor expression. Both the Conduit and the Life-Net CEL25 work in conjunction with the Cellect selective retention device to create an osteogenic graft material that is rich in cells and void of many of the morbidities associated with standard ABG harvesting procedures.

Similar devices such as the Wright Medical IGNITE Power Mix and techniques such as the graft reaming technique have recently been introduced; however, neither of these methods serves to concentrate bone marrow osteoprogenitor cells. The IGNITE system uses a similar bone marrow aspirate coupled with DBM but does not use a mechanism to concentrate any type of bone marrow cell. Moreover, the graft reaming technique takes a combination of bone marrow and bone graft reaming, necessitating a larger incision and thus higher degree of morbidity. Once again, this reaming technique does not incorporate a means by which cells are concentrated. A fundamental advantage of the Cellect system is its ability to use a larger volume of bone marrow from which osteogenic progenitor cells are isolated before implantation with TCP or DBM matrix.

**INDICATIONS AND CONTRAINDICATIONS**

The cell retention model can be used for osseous defects that occur to the extremities, pelvis, and spine due to trauma or surgical intervention. The Cellect system is indicated for the delivery of autograft, allograft, or artificial bone graft at an osseous surgical site.

This system is not to be used in patients that have fractures of the growth plate, segmental defects, fractures with insufficient vascular supply proximal to graft site, infection at the site of injury, or insufficiency in soft tissue coverage. Metabolic bone disorders or systemic diseases that effect bone healing are also relatively contraindicated with this system. This system may be used concurrently with a rigid fixation device or contained osseous defect.

Cell retention technology is designed to be used in conjunction with heparin and may be associated with bleeding or thrombocytopenia. Therefore, this system should not be used in patients that have heparin hypersensitivity.

**PREOPERATIVE PLANNING**

Potential limitations are noted in patients who would have a low volume of concentrated stem cells, which may be due to the age and sex of the patient as well as to systemic diseases that may be present. The issue of the relationship between “high risk” patients and number of osteogenic progenitor cells contained within an aspirate sample is a “double-edged sword” because these are the patients who would ideally benefit from this procedure.

The aging bone shows decreases in both mass and strength. Previous studies have documented a significant age-related decline in the number of nucleated cells harvested per aspirate for both men and women ($P = 0.002$). These progenitor cells may therefore be lacking in both quality and quantity. These findings suggest that patients with age-related bone loss and postmenopausal osteoporosis may not provide adequate numbers of cells.

Furthermore, studies also suggest that patients with systemic complications due to corticosteroid use or alcohol abuse have decreased activity of bone marrow cells. Likewise, patients who are managing chronic diseases such as diabetes mellitus are known to have a 40% decrease in DNA of collagen content of the callus, which correlates to callus cellularity. This decrease in DNA callus cellularity may be indicative of decreased concentration of bone marrow aspirate and an individual assessment should be considered with such patients.

**TECHNIQUE**

The following protocol for bone marrow harvesting and utilization is from DePuy’s manual for Cellect-Cell Capture Technology. Either an anterior or posterior approach may be used to harvest the aspirate. For an anterior approach, manually palpate to locate the anterior superior iliac spine and make a 2-mm incision with a #11 blade. The initial trajectory should be medially in line with the pelvic wing as gauged by the inner and outer tables. The needle should be aimed posterior to the iliac tubercle for entry into the medullary canal just beneath it. Gently tap the needle into the cortex of the bone and use a mallet to further the needle in the bone. Either 1 or 3 aspiration holes with needles can be used.

The posterior approach uses the same incision size and blade, yet the surgeon needs to palpate the patient to locate the superior aspect of the posterior superior iliac spine. The initial trajectory approach is approximately 40 degrees lateral from the parasagittal plane and 35 to 40 degrees inferior from transverse plane. Gently tap the needle into the cortex of the bone and use the mallet to further drive the needle in further.

A single-needle approach should be advanced 1 cm through the trajectory where 2 mL of marrow can be aspirated. Then advance another centimeter to take another 2 mL of aspirate. Continue this procedure until the syringe is full.

A triple-needle approach may also be used using either the anterior or posterior approach. Once the cortex
has been penetrated, continue to advance the needle until all 3 aspiration holes are engaged. Continue to pull back on the plunger of the syringe until 6 mL of aspirate is retrieved. Advance the needles 3 cm to pull another 6 mL of aspirate out. Remove the full syringe and replace it with new one. With this method, the needle can be advanced approximately 5 to 7 mL parallel to the iliac crest.

The Cellect protocol has a specified heparin to marrow ratio. The syringe should be prefilled with heparin at a concentration of 1000 U/mL. A 12-mL marrow aspirate should be added to the heparin. The heparin to marrow aspirate is the same for both the Conduit TCP granule method and the LifeNet CEL25 DBM method.

The matrix of choice (either TCP or DBM) needs to be prehydrated with saline for 1 minute before dispensing the bone marrow into the cartridge. After prehydration of the matrix, the marrow is dispensed within the cartridge. The selective retention-device syringe pump is activated when the pump handles are depressed. Two cycles of depressing the device are needed. After activation, the clotting ports are opened, and the bone marrow (or platelet-rich plasma, available commercially) and heparin are added to the system. Visible saturation is critical before using the material. Wait 2 to 3 minutes before removing the graft from the cartridge for implantation into the defect. The selective stem cell matrix is now ready for clinical application.

**POSTOPERATIVE MANAGEMENT**

The postoperative management after this procedure and fixation is the same standard of care as for any

![Image](image_url)
other fracture or arthrodesis. Monitoring for infection, immobilization, and fixation of the bone is essential. This system should only be considered if a rigid fixation is used and soft tissue covering is sufficient.

CASE REPORT
A 44-year-old woman with sickle cell disease presents with avascular necrosis of the talus, leading to severe ankle arthritis. The patient undergoes ankle fusion using a lateral approach and fibula sparing technique. Due to the higher risk of nonunion secondary to avascular necrosis, the ankle fusion is performed using rigid internal fixation in conjunction with SCR via Cellect using LifeNet DBM graft (Fig. 1). See also preoperative and follow-up images (see Fig. 2).

POSSIBLE CONCERNS, FUTURE OF THE TECHNIQUE
The study of bone biology has allowed the exploitation of cellular and molecular markers for better bone healing. Through the use of intraoperative concentration of autologous bone marrow aspirate, we have decreased the morbidity of iliac graft harvesting, eliminated the disease transmission from allograft, and eliminated adverse immunologic reaction due to donor rejection.

Future directives for this procedure may involve the use of growth factor augmentation including osteoinductive agents such as bone morphogenetic proteins, angiogenic signaling, or platelet-derived growth factor to activate the stem cell–concentrated graft and complex. This may be accomplished through staged reconstruction that comprised 3 steps: (1) concentration of the bone marrow...
aspirate using the Cellect protocol, (2) expansion of the progenitor cells in vitro with growth factor augmentation, and (3) finally implanting the modified and expanded cell line into the osseous defect.

Cellect is one of several commercially available bone marrow aspirate stem cell systems. It has not been determined clearly if one system has clinically significant advantages over another. However, minor subtleties exist between the different brands in terms of aspiration kits, and the Cellect selective retention device offers the added component of a concentration mechanism of an osteogenic progenitor cell. The iliac crest carries the greatest number of mesenchymal pleuripotential cells, when compared to other areas that contain marrow.

**ACKNOWLEDGMENTS**

This work was supported by funding from DePuy Orthopaedics, Inc, a Johnson & Johnson Company.

**REFERENCES**


