Clinical Use of Marrow Osteoprogenitor Cells to Stimulate Osteogenesis

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This review of 15 years research into various methods and techniques of using marrow osteoprogenitor cells shows that marrow grafts can be useful for numerous skeletal healing problems, but not all. The method offers considerable improvement over standard open iliac crest grafting and provides an attractive and advantageous method of stimulating osteogenesis in the management and prevention of nonunion.

The use of autologous bone graft to stimulate skeletal repair has been standard and effective since the work of Phemister reported more than 50 years ago and others as reported by Burwell. This author and many other investigators have not always been satisfied completely with this standard technique that requires surgical exposure of the donor site to obtain what often is a limited amount of graft and then a second surgical exposure to apply the graft to the recipient site. A significant number of complications can be the consequence at either operative location.

In this era of minimally invasive surgery for every system from the brain and heart to the hand and foot, a minimally invasive method of bone grafting should be an attainable goal. During the past 15 years, the author and coworkers have evaluated the use of marrow osteoprogenitor cells aspirated from the iliac crest and injected into the fracture site or area of skeletal defect to stimulate osteogenesis. This technique was found to provide a useful alternative to open grafting with considerable advantages. This article aims to summarize the experience and outline the rationale for using marrow osteoprogenitor cells to stimulate osteogenesis in numerous applications.

The fact that marrow can form bone has been known for more than a century but only recently has its clinical application been considered. The versatility of marrow as a transplant has been shown successfully for numerous hemopoietic diseases since the initial investigations for which Thomas received the Nobel prize in 1990.

As the mechanism of marrow transplants were studied and clarified, it became apparent that blood production requires a hemopoietic inductive microenvironment to support the manufacture of the 400 billion red cells and 10 billion white cells required daily. This microenvironment is provided by the stromal support system of marrow including the osseous trabeculae and other stromal cells in marrow.
The osteoprogenitor stromal system and hematopoietic system are mutually interdependent. Osteoblasts release monocyte and granulocyte stimulating factors, among others, which promote blood cell production. Conversely, the blood cell monocyte and osteoclast system releases factors from bone matrix that stimulate osteogenesis and are important for maintaining cortical and trabecular structures. Burwell, Urist, and others have elucidated this theory of medullary osteogenesis which includes bone inducing factors and reacting stem cells present in marrow.

In 1982 the author and coworkers began investigating the use of marrow osteogenesis in animal models of nonunion and clinical problems of fracture healing. Numerous animal studies have been reported elsewhere and will not be summarized here, except where they lead us to understand better the clinical use of marrow osteoprogenitor cells. However, without the initial animal studies, it is doubtful that investigators would have been encouraged to evaluate the use of marrow osteoprogenitor cells for numerous clinical applications.

INITIAL CLINICAL APPLICATION

The first clinical experience with percutaneous injection of marrow to stimulate fracture repair was reported in 1986 when a 31-year-old patient with an infected nonunion of the tibia was treated. This type of fracture might have been treated at that time with an open posterolateral grafting, but this patient was reluctant to undergo the open surgical procedure on his pelvis and draining fracture site. Consequently, the fracture site was treated by injecting 85 mm of autologous marrow aspirated from the patient's iliac crest in 5-mm aliquots. This material was injected directly into the posterior lateral aspect of the fracture without additional supplementation or carrier. The fracture was healed clinically and confirmed by radiographic evaluation 6 months after the injection (Fig 1).

Subsequent experiences with similar cases, including open Grade III tibial fractures treated in conjunction with either intramedullary rodding or plate fixation, showed autologous marrow injection to be as effective in stimulating osteogenesis as the standard technique of open iliac crest grafting. The experiences with the use of marrow grafts for osteogenesis from 1986 to 1995 were reported last year. This report summarized experience with 100 patients and indicated an 80% response to marrow grafts used in conjunction with adequate fracture stabilization. Conversely, the report also indicated that approximately 20% of patients had no or minimal bone forming response when treated with autologous marrow injection.

On additional investigation, it became evident that the ability to form osteoprogenitor colonies when marrow is cultured in vitro varies from species to species and from individual to individual. In marrow taken from 12 patients and studied in cultures, the ability to form osteogenic colonies varied widely, particularly when the marrow from older patients was compared with that from younger patients. This was evident from measuring the colony forming efficiency of marrow determined by the ratio of the number of bone forming colonies relative to the amount of cells cultured.

The ability to form osteoblastlike colonies in vitro can be affected by the techniques of marrow preparation and by the presence or absence of factors such as platelet derived growth factor, and particularly by blood loss. Lippiello et al found colony forming efficiency to be improved, at least temporarily, by a 1% loss of blood volume. This finding is consistent with the fact that blood loss causes a temporary elevation of erythropoietin, which in turn brings about an increased blood cell production. Work by Beck et al, Lippiello et al, Bab et al, and Einhorn et al indicates that there also is an increase in an osteogenic peptide circulating secondarily to blood loss or bone marrow extraction. This appears to be a bone stimulating peptide, closely resembling erythropoietin, that stimulates production of hemopoietic cells by the marrow. Thus, as the need for blood
Methods of Increasing Marrow Osteogenesis Efficiency

Bruder et al. and Haynesworth et al. showed that osteoprogenitor or mesenchymal stem cells can be increased significantly by various cell culturing techniques. These and other investigators have attempted to improve osteogenic efficiency by centrifugation techniques and by combining autologous marrow with carriers such as demineralized bone matrix. Tiedeman et al. found such composite marrow grafts to be particularly effective in a canine model of a gap nonunion. This study showed that a composite graft of autologous marrow and demineralized bone matrix produces mechanical and radiographic effects in the canine nonunion equivalent to those in open autologous grafting. Thus the use of composite marrow grafts clinically where there were problems of osseous defects that could not be filled adequately by marrow alone was encouraged.

In this technique, the marrow is aspirated in aliquots from the patient’s posterior iliac wing and mixed with demineralized bone matrix in a ratio of three volumes of marrow to approximately one volume of demineralized bone matrix. This provides a mechanically workable and biologically effective graft to pack into defects in unlimited amounts. Such a composite graft is considerably easier to use than the inconsistently sized grafts usually obtained from the iliac crest in limited amounts.

A preliminary study at Orlando Regional Healthcare System compared the clinical effectiveness of composite marrow grafts with both iliac crest grafting and allografts. To compare a standard clinical problem rather than fractures, which are nonstandard, the problem of posterior spinal fusion for progressive idiopathic adolescent scoliosis was studied. This comparison was done for 80 consecutive patients. All procedures were done by one surgeon using a standard technique of instrumentation with only one vari-
able: the type of grafting used for fusion. The patients all were followed up for a minimum of 2 years, and the outcome was evaluated on the basis of loss of scoliosis correction. This was considered to be indicative of slow or unsatisfactory fusion and represented a considerably more restricted criterion than most studies of scoliosis fusions based solely on the presence of pseudarthrosis.

Followup after scoliosis fusion ranged from 2 years to 5½ years. It was observed that the composite graft of bone marrow and demineralized bone matrix provided a rapid and successful spinal fusion equivalent to that of autologous iliac crest grafting and superior to that of allografting. The study concluded that in addition to its osteogenic effectiveness, the composite grafting technique reduces morbidity, blood loss, and operating time. Furthermore, the composite marrow grafting method provides an unlimited source of bone graft that can be worked readily into, over, and around the stabilization rods (Fig 2).

**USE OF AUTOLOGOUS MARROW TO TREAT FRACTURE GAPS CAUSING DELAYED UNION AND NONUNION**

In a review of 200 osseous healing problems during the past 20 years, it became apparent that initial fracture treatment is rarely a neutral factor without effect on the outcome. A primary cause of delayed union and nonunion, other than the initial injury inflicted on the bone, is the presence or absence of a fracture gap, which may result from bone lost at the time of fracture, excessive bone excision by the surgeon, or less than rigid internal fixation. Numerous investigators have shown that fracture gap is associated with normal osteoclastic bone resorption occurring the first few weeks after fracture. This initial osteoclastic bone resorption has been measured to occur 50 times faster than initial osteoblastic bone formation. The consequence is widening of the fracture gap seen on radiographs in the first few weeks after injury. Unfortunately, if treatment of the fracture maintains or encourages this gap, delayed union or nonunion commonly occurs.

In the past when fractures of the tibia, the most common site of nonunion, were treated by early weightbearing casts, the fracture gap usually was overcome by allowing slight fracture shortening. This also promoted callus by intermittent loading of the fracture in the cast. Similarly, when the unlocked Lottes nail was used to stabilize the fracture internally and supplemented with cast support, the fracture gap was eliminated by allowing the fracture fragments to slide over the nail.

The trend during the past 10 years toward using locked intramedullary nails for tibial fractures has provided better stabilization, but it also has created numerous problems resulting in a persistent fracture gap. Consequently, there is a need for early modifications of fixation to improve fracture stabilization and promote osteogenesis. This need can be met effectively by percutaneous marrow injection, particularly for fractures of the type most likely to produce problems of healing.

This concept of early grafting, advocated by Charnley 30 years ago, gradually has become accepted in the face of problems associated with either internal or external fixa-
tion techniques (Figs 3, 4). Charnley\textsuperscript{16} and others\textsuperscript{5,9} recommended early bone grafting by 6 to 12 weeks for fractures in which a fracture gap has become evident or for common Type II or Type III open fracture. The author and others have found that the fracture gap can be treated effectively by percutaneous marrow injection without the need to reopen the fracture site.\textsuperscript{8,19,25,30,62} This minimally invasive method of osteogenic stimulation provides effective solutions for overcoming bone gaps in conjunction with adequate mechanical stabilization of the fracture (Figs 3, 4).

**USE OF BONE MARROW GRAFTS FOR DEVASCULARIZED OR EXTRUDED BONE FRAGMENTS**

An important factor contributing to the development of a fracture gap has been aggressive operative debridement of bone. Aggression can be defined as a hostile act. Loose bone fragments, rather than being treated as hostile enemies, should be regarded as friendly allies of fracture repair. Even devascularized bone fragments are helpful in filling dead space at the fracture site and should not be considered enemies of fracture healing. The misconception that devitalized bone fragments should be excised aggressively has been prevalent in some recent publications on the subject of open fracture management.\textsuperscript{35,59} The technique even has been advocated,\textsuperscript{29} but should be challenged.

The concept that loose bone fragments should be excised conflicts directly with the experience of managing high velocity missile wounds in the Vietnam War. Studies\textsuperscript{40,65,69} of this experience indicate that excising multiple fracture fragments produced by severe military injuries created unnecessary fracture defects and inevitable healing problems. Moll and Willhoite\textsuperscript{40} reported experiences from Vietnam in which numerous problems of bone gap were created by aggressive surgical attacks on high velocity missile war wounds. Other investigators of wounds sustained in military action also described the importance of preserving bone in highly comminuted fractures.\textsuperscript{65,69} Subsequently, the North American Treaty Organization (NATO) handbook on wound debridement\textsuperscript{49} recommended avoiding excision of bone fragments. However, like much military experience, this quickly was forgotten by civilian surgeons who never had exposure to massive war casualties in unique military circumstances.

The consequence of needlessly excising bone fragments is the creation of defects that

**Fig 3A–B.** (A) Lateral radiograph of fractured femur 4 months after intramedullary nailing with a loose rod shows distal screw breakage and widened fracture gap. The fracture site remained painful for the patient, and additional treatment was advised. (B) Radiograph 2 months after re-nailing and percutaneous marrow injection shows callus formation, with the fracture gap obliterated. Pain from the fracture site had diminished, and the patient was walking without external support.
can be massive problems to overcome. This is seen particularly with comminuted fractures in the metaphyseal and articular regions where aggressive surgical debridement can leave insurmountable defects and unstable joints.

Occasionally, large gaps have resulted from ineffective use of the Ilizarov lengthening technique, which although helpful in many circumstances, can encourage needless excision of bone. The subsequently unhealed and nonfunctioning limb may be considerably worse than an amputation unless the massive osseous defect can be overcome.

According to the author’s experience, even extruded large condylar fragments or devascularized bone fragments can be scrubbed and sterilized sufficiently to allow reimplantation. These reimplanted fragments then can be supplemented by autologous bone marrow grafts to promote osseous union within the fracture bed. If the wound has not closed sufficiently at the time of initial treatment, autologous marrow injection can be delayed 4 to 6 weeks until the wound inflammation subsides.

The method of safely cleansing and sterilizing bone fragments has been reported by Van Winkle and Neustein, who recommend scrubbing the bone fragments, if contaminated, with chlorhexidine gluconate, and then soaking them in antibiotic solution for approximately 1/2 hour.

The rationale for recommending that devitalized bone fragments be discarded on debridement is based on fear of sequestrum forming and promoting infection. This, however, might be considered analogous to past fears of implanting hardware in an open fracture, which have been proven unfounded for most open injuries. It reasonably might be concluded that devitalized bone is no more detrimental than devitalized metal for wound healing and considerably better than dead space for fracture repair. Often it is necessary to combine the devitalized bone segment with devitalized internal fixation such as intramedullary nailing. Such segmental fractures can be stabilized successfully with an intramedullary rod to eliminate a fracture gap that would occur if the fracture fragment were excised.

The devitalized fragment can be stimulated to incorporate into the healing fracture by autologous marrow injection, as described by Healey et al and others have demonstrated that injection of autologous...
Fig 5A–B. (A) This open tibial fracture was treated with massive excision of bone fragments, and the patient subsequently was treated by the Ilizarov technique as illustrated here. (B) Radiograph showing the result 2 years after treatment. The patient still had a nonfunctioning limb with failure to heal.

Fig 6A–B. (A) In contrast to the problem of massive bone gap produced by surgical excision illustrated in Figure 5A and 5B, these radiographs show the result of comminuted Grade III open femur fracture, with the medial condyle extruded from the wound. The extruded bone was treated by sterile cleansing and soaking in antibiotic solution, as described by VanWinkle and Neustein.65 The fracture was immobilized with internal fixation and external cast brace support. Percutaneous marrow injection was performed at 4 weeks when the soft tissue wounds had healed. (B) Radiographs at 2 years show healing of the fracture despite the devascularized extruded condylar fracture. Some arthritis but no avascular necrosis is evident.

marrow aids repair of fractures in allografts. The use of autologous marrow with osteoprogenitor cells offers numerous options and considerable opportunity for additional improvements of technique. As more is learned about the physiology of osteoprogenitor cells and methods of maximizing their osteogenic efficiency, numerous additional clinical applications will become available.

In addition to developing newer techniques, surgeons must learn from past therapeutic mistakes. Bone gaps associated with either internal or external fixation or with overly aggressive surgical assault on the frac-
Fig 7A–C. (A) Initial radiograph of a segmental Grade III-B open tibial fracture shows a completely devascularized fracture fragment that was not discarded but used to fill in the fracture gap. (B) The segmental fracture and its devitalized fragment were immobilized with an intramedullary nail. In addition, bone marrow was used to stimulate osteogenesis, and healing occurred as seen on this radiograph at 12 weeks. (C) Bone scan of the segmental devascularized fracture at 2 weeks shows extent of devascularization of the fracture, with slow revascularization occurring.

ture should be avoided. When gaps do become evident, they can be corrected promptly with osteogenic stimulation by percutaneous marrow injection along with adequate mechanical stabilization of the fracture.

References
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