

Bone Marrow and Recombinant Human Bone Morphogenetic Protein-2 in Osseous Repair

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Bone marrow stem cells and recombinant human bone morphogenetic protein-2 each has the capacity to repair osseous defects. Recombinant human bone morphogenetic proteins require the presence of progenitor cells to function. It is hypothesized that a composite graft of recombinant human bone morphogenetic protein-2 and marrow would be synergistic and could result in superior grafting to autogenous bone graft. Syngeneic Lewis rats with a 5-mm critical sized femoral defect were grafted with recombinant human bone morphogenetic protein-2 and marrow, recombinant human bone morphogenetic protein-2, marrow, syngeneic cancellous bone graft, or

carrier alone (control). Serial radiographs (3, 6, 9, 12 weeks) and torque testing (12 weeks) were performed. Bone formation and union were determined. The recombinant human bone morphogenetic protein-2 and marrow composite grafts achieved 100% union at 6 weeks. Recombinant human bone morphogenetic protein alone achieved 80% union by week 12. Both groups yielded a higher union rate and superior mechanical properties than did either syngeneic bone graft (38%) or marrow (47%) alone. The superior performance of recombinant human bone morphogenetic protein-2 combined with bone marrow in comparison with each component alone strongly supports a biologic synergism. This experimentation shows the clinical importance of establishing operative site proximity for the osteoinductive factors and responding progenitor cells.

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List of Abbreviations Used

| | |
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| rhBMP | Recombinant human bone morphogenetic protein |
| mRNA | Messenger ribonucleic acid |
| BMP | Bone morphogenetic protein |

The repair of segmental osseous defects caused by trauma or tumor resection and spinal arthrodesis represents a challenge to

orthopaedic surgery. Each year in the United States, more than 250,000 procedures require the use of bone grafts to reconstruct skeletal defects.¹³ Autogenous cancellous bone graft is considered the gold standard for the treatment of bone deficit. The components required to heal fractures are provided naturally by autogenous cancellous bone graft. These basic components are osteoinductive growth factors, an osteoconductive hydroxyapatite and collagen matrix, and osteogenic stem cells present in bone marrow elements. Despite the effectiveness of autogenous bone graft, only a few donor sites in the skeletal system are suitable for supplying the material. Surgical morbidity, donor site pain, paresthesias, anesthesia, and infection are associated with autogenous bone graft harvesting (approximating 8% to 10%).⁵³ Allografts, as an alternative substitute to autogenous graft, are less desirable because of immunologic rejection, the potential of disease transmission, infections, and biologic inferiority.

To overcome these problems with autografts and allografts, investigators have attempted to develop synthetic composite grafts that are intended to mimic the natural course of components required by fracture healing. Developing a comparable bone graft substitute requires all of the components provided by autogenous cancellous bone. The relationships among osteoinduction, osteoconduction, and osteogenic stem cells are interdependent; each component requires the other two components to maximize bone forming ability. An idealized synthetic bone graft would contain: (1) osteoinductive growth factors to stimulate osteoprogenitor stem cells as signaling molecules; (2) primitive osteoprogenitor stem cells with receptors that respond to these signals by differentiating into osseous forming cells; and (3) osteoconductive material to provide a favorable environment for cells and growth factors to function. Such matrix should be replaced by newly formed bone with full integration into the host bone.

Among all of the growth factors that were tested in heterotopic and orthotopic locations, BMPs (either recombinant or extracted forms) are the most promising osteoinductive substances for bone formation.^{9,21,38} Several BMPs (hBMP, rhBMP-2, BMP-3 [osteogenin], BMP-4, and BMP-7 [osteogenic protein-1]), each as a single substance, have been shown to induce bone formation in several species using segmental defect models.^{3,8,9,12,16,19-21,32-34,37,38,40,52} Initial studies showed that BMP-2 (which is one of the most studied osteoinductive factors) has the capability of healing critical defects *de novo* in rat, sheep, dog, and primate models when it is combined with biodegradable polymers or guanidine hydrochloride extracted demineralized bone matrix as a carrier.^{1,9,12,16,52}

Because of immunogenicity, sterility concerns, and the risk of disease transmission, the use of inactivated demineralized bone matrix is limited in clinical situations. Different formulations of polylactic coglycolic acid, polylactic acid, and other biodegradable matrix candidates as carriers for BMPs have been tested in the authors' laboratory and by others.^{17,18,20,21,26,27,35,37,41,42} The authors' previous experience has shown that a synthetic bioerodible matrix composed of a copolymer of polylactide and polyglycolide porous microspheres was as effective as inactivated demineralized bone matrix when used as a carrier for rhBMP-2.^{20,21,27} Polylactic glycolic acid combined with rhBMP-2 successfully healed rat femoral defects and rabbit ulnar defects.

Bone marrow is a source of osteoprogenitor stem cells.^{5,6,7,11,14,15,22,23,30,38,47} Several investigators have shown successful healing of segmental bone defects using fresh bone marrow alone in various species.^{11,23,30,31,48} However, these experiments required large amounts of marrow to be implanted in the defects because of the minimal number of cells in the marrow that are capable of differentiating into osseous forming cells. The healing rate was correlated with the amount of marrow that was used. Embryonic studies

using *in situ* mRNA hybridization have shown a close geographic relationship between BMP production and receptor stem cells.^{25,28} These study findings indicate a synergism between the bone morphogenetic proteins and their responding cells.

The current investigation was designed to test the hypothesis that bone marrow osteoprogenitor cells and osteoinductive rhBMP-2 are synergistic, and a composite graft containing both components is superior to each alone and to cancellous bone graft.

MATERIALS AND METHODS

Animals

Ninety male syngeneic Lewis rats weighing 325 to 350 g were used for this experiment, and an additional 15 rats served as a source of cancellous bone and bone marrow. The test rats were maintained on rodent chow (Purina, St Louis, MO) and water *ad libitum* and caged in pairs. Unrestricted weightbearing and activity were allowed as tolerated after the operative procedure. This study was approved by The Institutional Animal Care Committee at The Hospital for Special Surgery.

Implant Materials

Bone Marrow

Bone marrow was harvested from the medullary cavity of the tibiae and femurs of the donor rats as described previously by Werntz et al.⁴⁷ The marrow was passed serially through 18- and 21-gauge needles to prepare isolated cells. Test groups using bone marrow had their defects filled with the marrow from one femur and one tibia (approximate volume 0.075 mL with 100×10^6 nucleated cells).

rhBMP-2

Recombinant human BMP-2 was obtained from Genetics Institute, Inc (Cambridge, MA). The preparation of rhBMP-2 has been described previously.^{44,45,51} Osteoinductive test groups were implanted with 9.3 μ g rhBMP-2 (Lot Number 2384-141 of rhBMP-2).

Polylactic Glycolic Acid

A 50:50 polylactic acid and polyglycolic acid copolymer preparation of porous microspheroids

(Lot Number 062491) was used as the carrier material (160 μ L/implant). The mean sphere diameter was 325 μ m (range, 3–500 μ m), and mean porosity was 70%.

Blood

Blood was obtained from the donor rats via cardiac puncture using a 25-gauge needle and tuberculin syringe (volume = 57 μ L/implant).

Cancellous Bone Graft

Bone graft was harvested from the metaphyses of donor syngeneic rat tibiae and femurs. The combined bone from one proximal and distal femur and one tibia was sufficient to fill each defect.

Operative Model

Rats were anesthetized with intramuscular ketamine (Ketalar, Parke-Davis, Morris Plains, NJ) (100 mg/kg body weight), and xylazine (Bayer, Shawny Mission, KS) (5 mg/kg body weight). Procaine penicillin SC (200,000 IU/kg body weight) was given intramuscularly for prophylaxis against infection.

The segmental defect model used has been described previously.^{29,47,48,52} A lateral approach to the femur was used, with all muscle and periosteal tissue circumferentially stripped about the diaphysis. A predrilled, high density polyethylene plate (23 \times 4 \times 4 mm), which was fabricated at the authors' institution, was fixed along the anterior cortex of the femur with 0.045-inch diameter flat ended threaded Kirschner (K) wires (Zimmer, Fort Washington, NY).

A 5-mm osseous defect (twice the diameter of the diaphysis) was created in the region of the middle of the shaft using a dental burr. Bone marrow was flushed from the medullary canal proximal and distal to the osteotomies with normal saline to simulate operative irrigation.

The implant materials were prepared at the time of the procedure. Lyophilized rhBMP-2 dissolved in the histidine and arginine buffer at a pH of 6.4 was mixed in polypropylene Eppendorf vials with 160 μ L of polylactic glycolic acid microspheres and 57 μ L of blood to form a congealed mixture. The preparation was allowed to clot for 30 minutes at room temperature and was chilled at 4° C for at least 15 minutes to allow clot retraction before implantation. The materials were placed within the defect site with the use of a spatula. The enveloping muscle enclosed the

implanted material, and the wound was closed in layers using chromic and nylon sutures.

Experimental Design

Seventy-five Lewis rats were divided into five test groups: (1) bone marrow and rhBMP-2 with polylactic glycolic acid; (2) bone marrow with polylactic glycolic acid; (3) rhBMP-2 with polylactic glycolic acid; (4) syngeneic cancellous bone; and (5) polylactic glycolic acid alone. Each test group contained 15 rats.

Serial radiographs were performed during a 12-week period to document progressive bone formation in the defects. Rats with fixation devices that failed within the first 3 weeks after surgery were excluded from the study. Data from rats with failure of the fixation devices after 3 postoperative weeks were included in the analysis.

All rats were sacrificed by asphyxiation with carbon dioxide at the conclusion of the study period. Both of the rats' femurs and the plates from the surgically treated legs were removed, and the femurs were tested mechanically. The involved and the contralateral femurs were tested to failure in torsion for mechanical verification of union.

ANALYSIS

Radiographic Evaluation

All rats were analyzed radiographically at 3, 6, 9, and 12 weeks after surgery. Under intramuscular sedation, each rat was positioned prone with the hindlimbs externally rotated (femurs parallel to the radiograph plate). Radiographs of both femurs of each rat were performed with a single contrast exposure of 50 kV, 4.9 mAs, 0.05 seconds, at a source target distance of 102 cm. The area of defect occupied by new bone was evaluated from the plain radiographs and recorded as a percentage of the total area of the defect for each rat at each point of analysis. A final radiograph was taken at the time of sacrifice at week 12.

Five observers blinded to the animals' implant material scored bone formation within the defect on a six-point validated scale as

follows⁵²: 0 = 0%, 1 = less than 25%, 2 = 25% to 49%, 3 = 50% to 74%, 4 = 75% to 99%, and 5 = 100%. Healing of the defects at each point was evaluated radiographically and confirmed by gross inspection and mechanical testing at the conclusion of the observation period. A defect was considered healed if osseous continuity was restored by greater than 25% of the osseous cross sectional diameter of the defect as determined radiographically (only bones with a 25% or greater union manifest a hard callus by the method of Nottebaert et al).²⁹

Mechanical Testing

Both femurs of all rats surviving at the conclusion of the 12-week observation period were harvested. Gross observations were recorded, and healed femurs were prepared. The soft tissue was stripped from the bone, and the plates were removed. Both femurs (the surgically treated and control ones) were mounted in a torsion testing apparatus (Burstein and Frankel⁴) with coaxial alignment of the defect maintained with the long axis of the torsion tester. Each femur was torqued in external rotation. The gross failure pattern for each surgically treated femur was assessed and classified into four groups according to the Nottebaert et al²⁹ modification of the biomechanical staging described by White et al⁴⁹ (1: nonunion; 2: union with failure within the graft; 3: union with failure across graft junction; 4: union with failure in the intact nongrafted bone) for fracture healing.

Statistical Methods

Five observers blinded to the animal's implant material scored the radiographs for bone formation and healing. An interobserver reliability study yielded an intraclass correlation coefficient of $r = 0.97$.^{36,52} Data on bone formation were analyzed using the Mann-Whitney test. The proportion of rats achieving union was compared radiographically and biomechanically between groups using Fisher's exact test. Tukey's nonpara-

metric independent group comparison analysis was used to evaluate the mechanical properties of the united defects.

RESULTS

Overall, six rats were excluded from the analysis: one each from the rhBMP-2 and bone marrow, and bone marrow groups and two each from the syngeneic bone graft and polylactic glycolic acid control groups. Two animals were excluded because of anesthesia related complications. Four rats had fixation failures at the first radiographic evaluation 3 weeks after surgery and were excluded from the analysis for perioperative technical fixation failures. No other fixation failures were seen throughout the 12-week observation period. No rat had an infection develop after surgery. Sixty-nine animals survived the 12-week observation period and were available for analysis. None of the control defects (13 of 13) implanted with polylactic glycolic acid healed (0%). Five of 13 (38%) rats in the syngeneic bone graft group, six of 14 (43%) in the bone marrow group, 12 of 15 (80%) in the rhBMP-2 group, and 14 of 14 (100%) in the rhBMP-2 bone marrow group experienced healing of their femoral defects by 12 weeks. In addition, the rhBMP-2 and bone marrow group had 100% union as early as 6 weeks.

Thirty-three pairs of united femurs were available for mechanical testing. No differ-

ences were seen in the observations by plain radiographs, high resolution faxitron images, gross inspection of healing, and mechanical testing for recognizing the femurs that achieved osseous union. The results for bone formation, healing, and mechanical testing for all groups were summarized and shown in Tables 1 to 3.

rhBMP-2 and Bone Marrow and Polylactic Glycolic Acid

By Week 3, 14 of the 14 (100%) rats in this group had at least 50% of the defect area filled with newly formed bone. By Week 6, the median area of the defect occupied by new bone was 100%. Bone remodeling and reconstitution of the medullary canal were evident radiographically at the conclusion of the 12-week observation period (Fig 1).

Seven of the 14 (50%) rats had healing of the defect radiographically at Week 3. By Week 6, 14 of 14 (100%) defects had evidence of healing, and by Week 9, all 14 animals had full osseous continuity restored.

All healed defects were confirmed on gross inspection at the conclusion of the 12-week study. The appearance of the defects at harvest paralleled the radiographic findings. Twelve of the 14 (86%) healed surgically treated femurs were evaluated mechanically in this test group. Two united specimens fractured at the drill holes during the harvest procedure and could not be tested biomechanically. The resultant spiral fractures

TABLE 1. Bone Formation: Area of Defect Occupied by Bone (Median Scores)

| Test Group | Week 3 | Week 6 | Week 9 | Week 12 |
|--------------------------|--------|--------|--------|---------|
| rhBMP-2/BM/PLGA (n = 14) | 3 | 5 | 5 | 5 |
| rhBMP-2/PLGA (n = 15) | 1 | 3 | 5 | 5 |
| BM/PLGA (n = 14) | 2 | 3 | 3 | 5 |
| ABG (n = 13) | 3 | 3 | 4 | 4 |
| PLGA (n = 13) | 0 | 0 | 0 | 0 |

Scoring system: 0 = 0%, 1 = < 25%, 2 = 25%-49%, 3 = 50%-74%, 4 = 75%-99%, 5 = 100%.

BM = bone marrow; rhBMP = recombinant bone morphogenetic protein; PLGA = polylactic glycolic acid; ABG = autogenous bone graft.

TABLE 2. Defects United by Time Point

| Test Group | 3 weeks | | 6 weeks | | 9 weeks | | 12 weeks | |
|-------------|---------|----------|---------|----------|---------|----------|----------|----------|
| | Union | Nonunion | Union | Nonunion | Union | Nonunion | Union | Nonunion |
| BMP/BM/PLGA | 7 | 7 | 14 | 0 | 14 | 0 | 14 | 0 |
| BMP/PLGA | 3 | 12 | 6 | 9 | 9 | 6 | 12 | 3 |
| BM/PLGA | 1 | 13 | 4 | 10 | 4 | 10 | 6 | 8 |
| ABG | 0 | 13 | 1 | 12 | 4 | 9 | 5 | 8 |
| PLGA | 0 | 13 | 0 | 13 | 0 | 13 | 0 | 13 |

| Time (weeks) | BMP/BM versus BMP | BMP/BM versus ABG | BMP versus ABG |
|--------------|-------------------|-------------------|----------------|
| 3 | p = 0.095 | p = 0.021* | p = 0.350 |
| 6 | p = 0.000* | p = 0.000* | p = 0.15 |
| 9 | p = 0.011* | p = 0.000* | p = 0.051 |
| 12 | p = 0.12 | p = 0.001* | p = 0.031* |

BM = bone marrow; rhBMP = recombinant bone morphogenetic protein; PLGA = polylactic glycolic acid; ABG = autogenous bone graft.

*Significant at p = 0.05 using Fisher's exact test.

showed two patterns of biomechanical failure; Nottebaert-Lane (modified White) Stages 3 (33%) and 4 (67%).

rhBMP-2 and Polylactic Glycolic Acid

By Week 3, five of 15 (33%) rats had new bone formation filling greater than 50% of the area of the defect. By Week 6, the median area of the defect occupied by new bone was 50% to 74%. Nine of 15 (60%) had complete fill of the defect with new bone by Week 9. Complete consolidation of the new and native bone was seen on radiographs in 12 of 15 (80%) rats by Week 12 (Fig 2).

Radiographic healing of the defects was evident in three rats (20%) by 3 weeks, 40% by 6 weeks, 60% by 9 weeks, and 80% by 12 weeks.

All radiographically healed defects were confirmed by gross inspection at the conclusion of the study. Ten (83%) of the healed surgically treated femurs were evaluated mechanically. Two united femurs were fractured at the drill holes during harvest and were unavailable for biomechanical testing. The median stage of healing as determined using the method of Nottebaert et al²⁹ was Stage 3.

TABLE 3. Modified White Scoring According to Nottebaert et al²⁹

| Test Group | I | II | III | IV | UF |
|-----------------------|----|----|-----|----|----|
| BMP/BM/PLGA (n = 14)* | 0 | 0 | 4 | 8 | 2 |
| BMP/PLGA (n = 15)* | 3 | 0 | 6 | 4 | 2 |
| Marrow/PLGA (n = 14) | 8 | 2 | 3 | 1 | |
| ABG (n = 13) | 8 | 2 | 3 | 0 | |
| PLGA (n = 13) | 13 | 0 | 0 | 0 | |

UF = united femurs fractured at the drill holes during harvesting; BM = bone marrow; rhBMP = recombinant bone morphogenetic protein; PLGA = polylactic glycolic acid; ABG = autogenous bone graft.

*p < 0.05 Tukey analysis versus ABG.

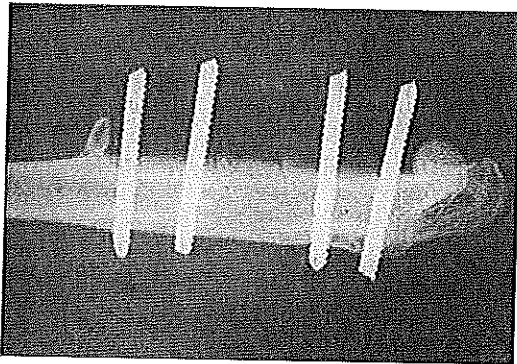


Fig 1. Radiograph of a femur 12 weeks after being implanted with rhBMP-2 and bone marrow and polyactic glycolic acid.

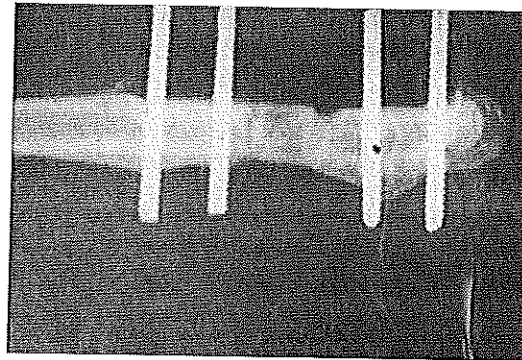


Fig 3. Radiograph of a femur 12 weeks after being implanted with cancellous bone graft.

Bone Marrow and Polyactic Glycolic Acid

By Week 3, the median bone fill was less than 50% of the defect area. Only seven of 14 (50%) animals had 100% bone filling by the end of the 12-week study (Fig 3).

At 3 weeks, one defect showed evidence of radiographic union; at 6 and 9 weeks 28% and at 12 weeks 43% of defects had progressed to union. Week 12 plain radiographs showed 6 (43%) defects had healed.

All six radiographically healed defects were confirmed on gross inspection at the conclusion of the study. The six (43%) animals with healed defects had three patterns of biomechanical failure: Stage 2 (33%), Stage 3 (50%), and Stage 4 (17%).

Syngeneic Cancellous Bone Graft

At Week 3, the median defect filled was less than 75%. The number of animals having progressive new bone formation did not increase until Week 9, when nine of 13 (69%) animals had greater than 75% of the defect area filled with bone. The entire defect was filled with new bone in six femurs (Fig 4).

No defect appeared healed radiographically at Week 3. One defect healed by Week 6 and 29% by Week 9. Five of 13 (38%) defects progressed to confirmed osseous union by Week 12.

Five of these healed surgically treated femurs and their matched contralateral femurs that were not treated surgically were evaluated mechanically. The resultant spiral frac-

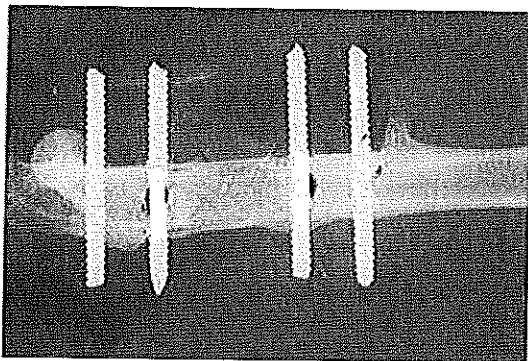


Fig 2. Radiograph of a femur 12 weeks after being implanted with rhBMP-2 and polyactic glycolic acid.

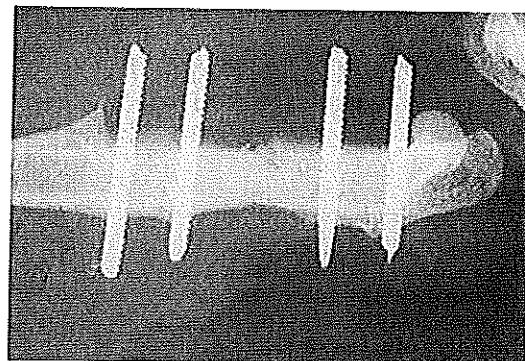


Fig 4. Radiograph of a femur 12 weeks after being implanted with bone marrow with polyactic glycolic acid.

tures of the five united defects had two patterns of biomechanical failure: Stage 2 (40%) and Stage 3 (60%).

Polylactic Glycolic Acid

Only minimal new bone formation was observed in this group. No rat had greater than 50% defect fill with new bone by 12 weeks (Fig 5). None of the defects progressed to osseous union, either radiographically or grossly, during the 12-week study.

Comparisons Between Groups.

The rhBMP-2 and bone marrow composite graft group had a significantly greater union rate than did the syngeneic bone graft, bone marrow, and polylactic glycolic acid carrier groups at all points (Table 2). The number of the united defects increased significantly in both rhBMP containing groups at 6 and 9 weeks.

The rhBMP-2 groups had a significantly higher number of defects that united than did the autogenous cancellous bone graft and the bone marrow group, as determined radiographically and mechanically at week 12 ($p = 0.05$). The bone marrow and the syngeneic bone graft groups were statistically comparable at all points (3, 6, 9, and 12 weeks).

The grafted bones were divided into the following groups by torque testing: non-union, union with failure through the defect,

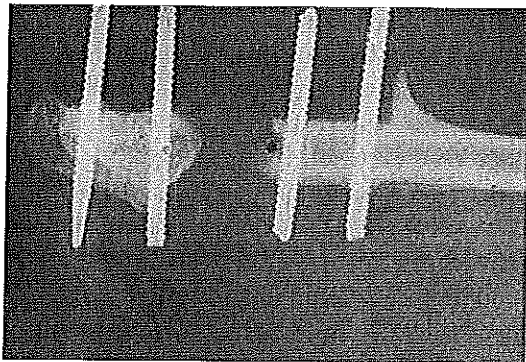


Fig 5. Radiograph of a femur 12 weeks after being implanted with polylactic glycolic acid alone.

union with failure partially through the graft junction, and union with failure through the intact nongrafted bone (Table 3). When comparing the various grafting techniques, the rhBMP-2 and bone marrow and rhBMP-2 groups were indistinguishable. Both rhBMP-2 groups had better results than the syngeneic bone graft group at 12 weeks using the Tukey nonparametric independent group comparisons with $p < 0.05$ (Table 3) that applied to the stages of Nottebaert et al.²⁹

DISCUSSION

This study radiographically and biomechanically showed that composite graft consisting of bone marrow and rhBMP-2 in a bioresorbable polylactic glycolic acid carrier effectively led to new bone formation and union. A rapid rate of osseous healing was achieved successfully using composite graft in an acute critical segmental rat femoral defect model. The experiment showed that osteoinductive rhBMP-2 and osteoprogenitor bone marrow cells in combination are significantly synergistic and superior to each alone and result in significantly better healing compared with autogenous cancellous bone graft.

Autogenous bone graft is considered the gold standard because it contains osteoinductive, osteoconductive, and osteogenic elements. When it is implanted into osseous defects and sufficient quantities are obtained, autogenous bone provides a minimal amount of bone marrow and a degree of osteoconductivity. During the remodeling process, this provides some osteoinduction by releasing the growth factors in its matrix. However, a limited amount of each element is present in the graft.

The fate of syngeneic cancellous bone graft in this model has been variable. Healing defects after bone grafting in previous studies has ranged from 25% to 82%.^{11,16,47,48} No previous study has shown that cancellous bone graft assured healing of the defects during the 12-week period. Syngeneic cancel-

lous bone graft performed poorly by all parameters in this study in comparison with grafts containing rhBMP-2. Only five of 13 (38%) cancellous grafted defects healed by 12 weeks. Cancellous bone grafting of human defects 6 cm or longer has marginal success.⁴⁶ The 5-mm defect in the rat is of comparable dimension with the additional insult of the removal of all periosteum. The extent and character of the rat defect in part explains the 38% union rate at 12 weeks with this form of grafting.

Previous studies by many investigators using different species and animal models, *in vivo* and *in vitro*, have shown that bone marrow has osteoprogenitor stem cells.^{11,14,22,23,30,38,47} However, the number of these cells in marrow is minimal (1/50,000 to 1/2,000,000).⁵ Healing segmental defects and nonunions using marrow implantation required a large volume of marrow.^{6,7} Bone marrow also does not provide good osteoconductive properties. The success of bone marrow in healing segmental bone defects was limited as a single adjuvant in these studies. In this study, bone marrow and poly(lactic glycolic acid) containing grafts healed approximately 50% of the defects at 12 weeks.

Poly(lactic glycolic acid) has been used as an effective carrier of BMP in a rabbit ulnar defect model and canine radial defect model, and it was found to be effective.^{3,16} Studies by Kenley et al²⁰ and Lee et al²¹ showed comparable results when using similar poly(lactic glycolic acid) preparation as a carrier for rhBMP-2 to induce healing in rat calvarial and segmental long bone defects. No adverse systemic effects were observed in the animals implanted with this preparation of poly(lactic glycolic acid). In addition, although the response was not directly evaluated, no appreciable detrimental local tissue response to the poly(lactic glycolic acid) implants was detected clinically. The mechanical properties of the femoral defects grafted with the poly(lactic glycolic acid) carrier were not compromised in this study or in a previous investigation by Bostrom et al.³

All rhBMP-2 and poly(lactic glycolic acid) grafts confined their newly formed bone within the defect. No ectopic bone formation was observed. New bone was evident radiographically by 3 weeks within the defects implanted with the composite rhBMP-2 containing material. The extent of the new bone formed was variable (range, 25%–100%); however, abundant new bone formation was evident radiographically by 9 weeks in all defects implanted with rhBMP-2. Consolidation of the bone formed within the defect, the development of a neocortex, and reconstitution of the medullary canal were observed consistently from the radiographs in these animals by 12 weeks. Approximately 80% of the segmental defects healed with composite implants of rhBMP-2 and poly(lactic glycolic acid) (without bone marrow) by 12 weeks.

An immunohistochemical study by Bostrom et al² showed that BMPs exist in the early phase of fracture healing. This study suggested that BMPs are one of the necessary elements for fracture healing. Additional evidence for BMPs' roles as signaling molecules or differentiating factors on multipotential stem cells comes from an *in vitro* study by Wang et al.⁴³ Their study showed that established mesenchymal stem cell lines (C3H10T1/2 and 3T3) can be differentiated into osteoblastlike cell when stimulated by rhBMP-2. The result of these studies indicates that rhBMP-2 can cause differentiation of a stem cell line into osteoblast phenotype.

In the current study osteogenic bone marrow combined with osteoinductive rhBMP-2 in an inert, bioerodible, carrier poly(lactic glycolic acid) showed the superior bone repair. These observations are consistent with other studies when bone marrow was combined with various components of bone matrix and other osteoconductive materials.^{11,15,23,27,30,38,47,50} In this experiment, combination of rhBMP-2 and bone marrow appeared to be synergistic, and it filled and successfully united all of the defects as early as 6 weeks. Although the rhBMP-2 in the absence of bone marrow can lead to successful

healing through the stimulation of local stem cells, the coinsertion of osteoprogenitor cells known to respond to osteoinductive growth factors significantly enhanced the bone repair process. In comparison with any other group used in this experiment, including cancellous bone grafts, the presence of osteoprogenitor cells from marrow combined with rhBMP-2 provided more rapid, predictable, and statistically superior results.

Although rhBMP-2 and marrow each independently can lead to successful repair, the combination of osteoinduction and osteoprogenitors is synergistic. This study suggests that introduction of the stem cells via bone marrow to the rhBMP-2 lead to the superior osteoinduction than rhBMP-2 alone. However, in a compromised bed where stem cells may be lacking or poorly functioning, such as occurs with radiation, the coinsertion of fresh marrow or the proximity of a viable muscle flap with stem cells may be critical.

A synthetic composite graft material composed of an osteoinductive growth factor and an osteoconductive matrix, both of potentially unlimited quantity, represents a favorable bone graft substitute for use in clinical situations requiring new bone formation. However, the addition of an easily obtainable source of osteoprogenitor cells (fresh bone marrow) to these components will enhance the rate of bone formation, increase percentage of successful union, and decrease the recovery time clinically. These advantages of this composite graft may be a practical solution to the difficult problems of managing bone loss. This study showed the clinical importance of establishing proximity for the osteoinductive factors and responding progenitor cells in the bone graft site.

Repair of the segmental osseous defects caused by trauma or tumor resection represents a challenge to orthopaedic surgery because autogenous graft is not readily available in large quantity. Different formulations of ceramics, preparations of collagen, and demineralized bone matrix with marrow have been tested to substitute for

autogenous bone graft in several animal models.^{1,10,13,22-24,30,31,38,39,48} However, in the absence of osteoinductive stimulants, only limited success was achieved with such studies.

The current study shows that using bone marrow as a source of osteoprogenitor cells combined with rhBMP-2 resulted in predictable, rapid bone formation and union using a rat segmental defect model. The composite graft consisting of rhBMP-2 and bone marrow was superior to each alone and was synergistic in producing bony unions. The particular preparation of polylactic glycolic acid that was used in this study appeared to be a suitable delivery vehicle for rhBMP-2 and bone marrow combination. A composite synthetic bone graft using osteoinduction, osteoprogenitor cells, and biodegradable osteoconduction showed synergism among the components and was superior to the gold standard autogenous bone graft.

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