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Radiomorphometry and Biomechanical Assessment of Recombinant Human Bone Morphogenetic Protein 2 and Polymer in Rabbit Radius Osteotomy Model

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Abstract: The study objective was to determine the mechanical integrity and radiopacity of regenerated bone within critical-sized defects (CSDs) in radii of rabbits using recombinant human bone morphogenetic protein 2 (rhBMP-2) with a porous, biodegradable poly(D,L-lactic acid) (PDLLA) carrier (designated PLA). Twenty millimeter, unilateral radial osteotomies were created in 96 skeletally mature New Zealand white rabbits. The rabbits were randomly assigned to six treatment groups with two euthanasia periods. Treatment groups included unfilled defect ($n = 8$), segmental autograft ($n = 8$), PLA + 0 μg rhBMP-2 ($n = 8$), PLA + 17 μg rhBMP-2 ($n = 8$), PLA + 35 μg rhBMP-2 ($n = 8$), and PLA + 70 μg rhBMP-2 ($n = 8$). The radiopacity was significantly greater for the 35- and 70- μg rhBMP-2 groups at 4 weeks compared to unfilled controls, PLA only, and 17- μg rhBMP-2 groups and equivalent to the autograft. At 8 weeks all groups receiving rhBMP-2 were equivalent to the autograft and significantly greater than unfilled defects and PLA alone. Similarly, the biomechanical analysis indicated significantly greater torque at failure for the 35- μg rhBMP-2 group compared to all other groups at 4 weeks. By 8 weeks all groups receiving rhBMP-2 and autograft had significantly greater torque than unfilled controls and PLA alone. These radiomorphometric and biomechanical results indicate PLA may be a suitable carrier for rhBMP-2 used for skeletal regeneration.

Keywords: bone morphogenetic protein; bone regeneration; critical size defect; rabbit; biomechanics

INTRODUCTION

Bone grafts have been used for decades to repair osseous discontinuities resulting from trauma, neoplasm resection, and congenital abnormalities; and over 100 000 are performed each year in the United States.^{1,2} Autografts are most widely used by surgeons. These grafts contain viable cells, bone marrow containing osteoprogenitor cells, and a collagenous matrix that combine to form a favorable milieu for osteogenesis, osteoinduction, and osteoconduction. The

major disadvantages of autografts are donor site morbidity and limitations on the quantity of graft material.^{1,3,4} Allografts are often considered as an alternative to autografts. Although allografts do not contain viable cells at the time of implantation, they serve as a scaffolding for revascularization and as a source for healing factors promoting osseous ingrowth (i.e., osteoconduction). However, allografts have been associated with disease transmission and infections.^{1,5-7} Additionally, common problems with allografts include nonunions and delayed unions.^{1,8} Gross et al.⁹ reported incomplete incorporation of allografts even as late as 7 years. Neither autograft nor allograft provide the patient with ideal bone graft material. Consequently, there is a strong clinical need to develop alternatives to autografts and allografts.^{1,2} Hydroxyapatite (HA), tricalcium phosphate, and bioactive glasses have proven to be viable bone

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Figure 1. A graphical representation of the temporal changes and comparative changes in radiopacity between treatment groups in the regenerating rabbit radii. The autograft and all rhBMP-2 filled defects produced significantly more bone than unfilled defects and PLA-only groups by 4 weeks. The radiopacity measurements represent the mean of eight animals for each treatment group. A, unfilled defect; B, segmental autograft; C, PLA only; D, 17 μ g rhBMP-2 + PLA; E, 35 μ g rhBMP-2 + PLA; F, 70 μ g rhBMP-2 + PLA. (*) B, D, E, F > A, C ($p < 0.05$). (**) B > A, C, D, E, F ($p < 0.05$).

graft substitutes.¹⁰⁻¹⁶ These alloplastic materials vary in porosity, granularity, size, and resorbability and typically serve as a template or scaffold for osteoconduction within the bony defect. More recently, recombinant genetically engineered bone growth proteins, such as bone morphogenetic proteins (BMPs) and transforming growth factor β (TGF β), were combined either with bioresorbable polymers or calcium phosphate carriers and emerged as an effective and promising osteoinductive bone graft alternative.¹⁷⁻²⁰ Animal studies validated the efficacy and safety of BMPs in regenerating non-load-bearing skeletal defects.^{18,21-24} However, delivering the protein to skeletal defect sites through use of an appropriate carrier has been a challenging task. The most common BMP carriers investigated include collagen sponges^{21,22} and biodegradable polymers.^{25,26} This study will use a biodegradable polymer [poly(D,L lactic acid) (PDLA) referred to as recombinant human BMP-2 (rhBMP-2) + PLA] adsorbed with rhBMP-2 as a potential bone graft. Therefore, the purpose of this study was threefold: to regenerate critical-sized defects (CSDs) in radii of rabbits with several varying doses of rhBMP-2 + PLA, to assess radiographic density and me-

chanical integrity of the regenerated bone, and to compare the rhBMP-2 regenerated bone to that of unfilled defects and autograft-filled defects. The hypotheses of the study were that one of the doses of rhBMP-2 + PLA would promote radiographic density similar to that of the "gold standard" autograft and the biofunctionality promoted by one of the rhBMP-2 doses would be equivalent to that promoted by the autograft.

MATERIALS AND METHODS

Ninety-six skeletally mature New Zealand white rabbits were randomly assigned to six treatment groups and two euthanasia periods (4 and 8 weeks). Treatment groups included

1. unfilled defect ($n = 8$),
2. segmental autograft ($n = 8$),
3. PLA + 0 μ g rhBMP-2 ($n = 8$),
4. PLA + 17 μ g rhBMP-2 ($n = 8$),

Figure 2. Photographs illustrating changes in radiopacity between treatment groups at 8 weeks including the unfilled defect (CSD), segmental autograft, 0 μ g rhBMP-2 + PLA, 17 μ g rhBMP-2 + PLA, 35 μ g rhBMP-2 + PLA, and 70 μ g rhBMP-2 + PLA.

5. PLA + 35 μ g rhBMP-2 ($n = 8$), and
6. PLA + 70 μ g rhBMP-2 ($n = 8$).

The PLA carrier used for rhBMP-2 was PDLA.

PDLA was designed with high porosity to enhance adsorption of rhBMP-2 within the macrostructure.²⁷ The polymer was manufactured into cylindrical formats (4.68 $\times$ 20 mm). Highly purified rhBMP-2^{18,28} was placed in sterile glass vials containing a sodium glutamate buffer (5 mM, pH 4.5). At the time of surgery, 0.17 mL of buffer containing either 0, 17, 35, or 70 μ g of rhBMP-2 was transferred in a sterile fashion by syringe to the sterile PLA delivery vehicle.²⁹ Immediately following the transfer of the growth factor the rhBMP-2 + PLA was placed into the osteotomy gap.

The rabbits were anesthetized using a mixture of 10 mL of ketamine hydrochloride (Ketaset, USP, 100 mg/mL, Fort Dodge Laboratories, Inc., Fort Dodge, IA) and 5 mL of xylazine (Gemini SA, 20 mg/mL, Burns Veterinary Supply, Inc., Rockville Center, NY) at a dose of 0.65 mL/kg body weight. Triple antibiotic ophthalmic ointment (Lacrilube, Allergan Pharmaceuticals, Irvine, CA) was applied to the rabbit's eyes when anesthetized to prevent dryness. Prophylactic antibiotics (Baytril, Miles Inc., Shawnee Mission, KS) were administered preoperatively at a dose of 2.25 to 2.50 mg/kg. The operative site (the left or right front limb) was shaved, prepped, and draped for aseptic surgery with the rabbit in the supine position. A tourniquet was placed around the axillary region. The subcutaneous region around the operative site was injected with 0.5 mL of 2% Lidocaine with epinephrine 1:100,000 (Xylocaine, 20 mg/mL, Astra Pharmaceutical Products Inc., Westborough, MA). An approximately 3-cm long superomedial

incision was made and the tissues overlying the distal diaphyseal radius were dissected. A 2-cm segmental defect was created in the radius with a mini-oscillating saw using copious sterile saline irrigation. The assigned treatment material was placed in the bony defect. No internal fixation was supplied because of the stabilization of the adjacent ulna. The soft tissues were closed in layers using 4-0 Vicryl sutures (Ethicon Inc., Somerville, NJ). The skin was closed with surgical staples (Precise DS-25, 3M, St. Paul, MN). Postoperative analgesia (Buprenex, 0.3 mg/mL, Reckitt and Coleman Pharmaceuticals Inc., Richmond, VA) was given as needed at a dose of 0.1 mL/kg. Water and food were provided *ad libitum*.

Immediate postoperative lateral radiographs were taken of the operative limb. Standardized radiographs were taken immediately postoperatively, every 2 weeks in life, and at sacrifice.

At sacrifice, the radius-ulna complex was disarticulated at the elbow and wrist and specimens were stored at -20°C with tissue intact until torsional mechanical testing was initiated. Torsional tests were preferable to bending tests in this animal model in that they simulate clinically relevant fracture patterns, apply equal loads to all sections of the bone, and are relatively insensitive to specimen length and orientation.^{30,31} Bone fails in torsion at the weakest point along the shaft.

Specimens were thawed to room temperature, the soft tissue was carefully removed, and the proximal and distal ends of the radius-ulna complex were potted in dental plaster (0-67 Snow White Stone, Miles Inc., South Bend, IN) in order to grip the specimen for testing. Transcortical cuts were made in the ulna adjacent to the potted ends to eliminate the mechanical contribution of the ulna and isolate the radius during torsional testing. Separation of the ulna from the radius was not possible due to ulnar hypertrophy, scar tissue formation, and fibrous fusion. Unpublished preliminary experimentation indicates the discontinuous ulna segment does not affect the breaking torque or stiffness. The radius of the specimens was loaded to failure in torsion using a biaxial MTS MiniBionix materials testing machine (MTS Inc., Minneapolis, MN) at a loading rate of $180^{\circ}/\text{s}$.³² Data were collected at 1000 Hz for the duration of the test. Values of ultimate torque (torque at failure) and angular displacement at failure were extracted from the data. Specimen stiffness and energy absorbed during failure were calculated as the slope of the torque/angle curve and the area under the torque/angle curve, respectively.

The biomechanical and radiomorphometric results were analyzed using a 2-way analysis of variance (ANOVA) to look at the effects of time (4 and 8 weeks) and treatment (A, B, C, D, E, and F). If significance was noted with the ANOVA, a 1-way ANOVA was used to look at the effects of time by treatment and treatment by time followed by a Duncan's multiple comparison procedure with a significance level set at $p = 0.05$. Given the sample size, the

Figure 3. Graphical representation of the breaking torque of the regenerating radii at 4 and 8 weeks. The 35- μ g rhBMP-2 was significantly greater than all other treatments at 4 weeks, yet by 8 weeks the autograft and all BMP-2 treated groups were equivalent. The breaking torque measurements represent the mean of eight animals for each treatment group. A, unfilled defect; B, segmental autograft; C, PLA only; D, 17 μ g rhBMP-2 + PLA; E, 35 μ g rhBMP-2 + PLA; F, 70 μ g rhBMP-2 + PLA. (*) E > A, B, C, D, F ($p < 0.05$). (**) B, D, E, F > A, C ($p < 0.05$).

statistical power of the analysis was 80% (SAS, Inc., Version 6.11, Cary, NC).

RESULTS

Radiomorphometry

Radiomorphometric analysis indicated the unfilled defect (group A) displayed radiolucent nonunion throughout the 8-week study. Autograft sites revealed a radiolucent interface bracketing the graft at 4 weeks that was less evident by 8 weeks. A slight radiopacity proximal and distal to a radiolucent midportion was observed for the unfilled defects (group A) and the 0- μ g rhBMP-2 + PLA cohort (group C) at 4 and 8 weeks. The autograft and all rhBMP-2 filled defects (groups B, D, E, and F) produced equivalent amounts of regenerated bone by 4 weeks that was significantly more bone than the unfilled defects and PLA-only groups (groups A and C) ($p < 0.05$). The same pattern

(B, D, E, F > A, C) was observed at 8 weeks (Fig. 1). All rhBMP-2 treated and autograft treated wounds revealed complete bridging and complete radiopacity of the bridging tissue by 8 weeks. A time-dependent effect was noted in the increase of opacity between the 2-week measurement and the 4-, 6-, and 8-week measurement for the autograft and all rhBMP-2 groups (groups B, D, E, and F) ($p < 0.05$). The unfilled defect and PLA-only group (groups A and C) did not exhibit more opacity with time. The temporal changes in radiopacity can be visualized in the graph of Figure 1 and the composite radiographs of Figure 2. PLA is radiolucent; therefore, the radiopacity of all groups except the segmental autograft group (group B) were equivalent immediately postsurgery. At 2 weeks the 35- and 70- μ g rhBMP-2 groups (groups E and F) portrayed a hint of radiopacity that increased and was equivalent to the autograft (group B) by 4 weeks. At 6 weeks the highest doses of rhBMP-2 (groups E and F) portrayed a bonelike radiographic quality, yet by 8 weeks complete bridging

Figure 4. Maximum angular displacement at failure of the regenerating radii at 4 and 8 weeks. All treatment groups were equivalent at 4 weeks. Yet, by 8 weeks the angular displacement of the PLA-only group was significantly greater than other groups. The angular displacement measurements represent the mean of eight animals for each treatment group. A, unfilled defect; B, segmental autograft; C, PLA only; D, 17 μ g rhBMP-2 + PLA; E, 35 μ g rhBMP-2 + PLA; F, 70 μ g rhBMP-2 + PLA. (*) C > A, B, D, E, F ($p < 0.05$).

was noted including cortical development. Groups A, C, D, E, and F were noted to have fringes of radiopacity that were localized near the host bone junction with no isolated radiopaque areas distant from the host bone.

Biomechanics

The failure torque was significantly greater for the 35- μ g BMP treated animals (group E) than for all other treatments at 4 weeks. By 8 weeks the autograft (group B) and all BMP treated animals (groups D, E, and F) had equivalent breaking torque, yet the unfilled defects and PLA-only defects (groups A and C) were significantly weaker (Fig. 3).

The angular displacement at failure was not different among the groups at 4 weeks. Yet by 8 weeks the angular displacement of the PLA only (group C) was significantly greater than all other groups (Fig. 4).

The mechanical stiffness of the defects at 4 weeks was equivalent for all rhBMP-2 treated groups (groups D, E,

and F) and the autograft group (group B) and significantly greater than the unfilled defects and PLA-only groups (groups A and C). Although the magnitude of stiffness was greater at 8 weeks for all groups, the same trends were noted (Fig. 5).

The energy absorbed at failure was significantly greater for the 35- μ g rhBMP-2 + PLA group (group E) than all other groups at 4 weeks but not greater than the autograft-filled defects (group B) ($p < 0.05$). However, by 8 weeks the energy to failure for the autograft group (group B) was significantly greater than all other groups. (Fig. 6).

DISCUSSION

The results of this radiomorphometric and biomechanical study concur with our hypothesis: all doses (17, 35, and 70 μ g rhBMP-2), used in conjunction with a PLA carrier, were able to regenerate bone in a rabbit radius CSD with comparable mechanical strength and radiopacity to auto-

Figure 5. Mechanical stiffness of the regenerating radii at 4 and 8 weeks. At both 4 and 8 weeks, all rhBMP-2 treated groups and autograft had significantly greater stiffness than unfilled defects and PLA-only groups. The stiffness measurements represent the mean of eight animals for each treatment group. A, unfilled defect; B, segmental autograft; C, PLA only; D, 17 μ g rhBMP-2 + PLA; E, 35 μ g rhBMP-2 + PLA; F, 70 μ g rhBMP-2 + PLA. (*) B, D, E, F > A, C ($p < 0.05$).

graft-filled defects within 4 weeks of surgery. The rhBMP-2 augmented defects healed significantly better than unfilled defects or defects filled with only PLA. There was a dose-response relationship of rhBMP-2 for radiopacity at 2 weeks, and the higher doses produced superior bone formation than the lowest dose. The results of this biomechanical study support our previously reported quantitative histomorphological findings indicating that rhBMP-2 and PLA carrier can effectively regenerate bone in a dose-response fashion at specific skeletal sites (rabbit radius).²⁹

The classic dose-response relationship was observed radiographically and biomechanically in rhBMP-2 treated animals, especially at later time points. At early time points the response was more variable. The actions of BMP are receptor mediated that would theoretically lead to a standard dose-response curve (plateau in response once the receptors are saturated). The temporal aspect of this study complicates the dose-response mechanism. In this *in vivo* bone regeneration system, BMP also has autocrine and paracrine functional components. The transient disparity

between the 35- and 70- μ g rhBMP-2 groups at 4 weeks seen in the biomechanical data, and to a lesser extent in the radiographic data, may simply be a stabilization process of the physiological system. At the later time points, 6 and 8 weeks for radiographic data and 8 weeks for biomechanical data, the data follows classic dose-response behavior. In a separate experiment, a similar rhBMP-2 dose-response was observed in the quantity of bone within the defect site that was assessed histologically.²⁹

The rhBMP-2 + PLA animals exhibited a significant time-dependent increase in bone formation (radiopacity) within the gap between 2 and 4 weeks. After 4 weeks no significant increase in bone within the defect was radiographically noted for any experimental groups. This would indicate that rhBMP-2 effects are seen immediately by stimulating early bone regeneration. The effects of local administration of rhBMP-2 would tend to diminish with dissolution of rhBMP-2 concentration at the defect site. Although the mechanical strength at 2 weeks was not evaluated, it would be estimated to parallel the increase in radio-

Figure 6. Energy absorbed at failure of the regenerating radii at 4 and 8 weeks. The 35-mg rhBMP-2 group had significantly higher energies at failure than all other treatment groups at 4 weeks except the autograft group. However, by 8 weeks the autograft group had higher energies than all other treatment groups. The energy measurements represent the mean of eight animals for each treatment group. A, unfilled defect; B, segmental autograft; C, PLA only; D, 17 µg rhBMP-2 + PLA; E, 35 µg rhBMP-2 + PLA; F, 70 µg rhBMP-2 + PLA. (*) E, B > A, C, D, F ($p < 0.05$). (**) B > A, C, D, E, F ($p < 0.05$).

capacity. Histological assessment of bone formation rates within the defect site would also confirm the time-dependent effects of rhBMP-2 noted radiographically and is planned for follow-up studies.

Although the use of rhBMPs for bone regeneration and developmental biology have been studied extensively,^{24,33} the use of PDLLA as a carrier for growth factors and/or an osteoconductive template has not been previously investigated. An appropriate delivery system is crucial to the overall effectiveness of the administered growth factor. The delivery system is responsible for the localization of the growth factor, mesenchymal cell deposition and retention, and establishment of a template for contour and structural confinement of new bone growth.

In previous studies two classes of delivery systems for rhBMPs were used: collagen based and poly(α -hydroxy acids).^{21,22} HA, HA-tricalcium phosphate, and demineralized bone matrix were also used to deliver rhBMPs.^{16,18} Calcium phosphate delivery systems are either not biodegradable or do not biodegrade at the same rate at which

bone is formed and therefore would have limited clinical utility. Demineralized bone matrix produced inconsistent clinical results due to infection, immunologic rejection, viral transmission, varying processing techniques and sterilization, and lack of continuity and structural integrity.^{34,35} There has been controversy over the biocompatibility and use of poly(lactic acid) and poly(glycolic acid) within skeletal defects.^{36,37} However, the material characteristics of these polymers holds great potential for use in skeletal regeneration, specifically their ability to degrade in a reproducible and designated fashion (given the molecular weight of polymeric components) with the potential for drug delivery release with degradation. The polymer used for this project, PDLLA (designated PLA) was custom designed to have specific characteristics of degradation kinetics to release rhBMP-2, which coincided with tissue regeneration within the defect.³⁸

The mechanical strength of regenerated bone using polymer carriers and rhBMP-2 was recently published by Bostrom et al.³⁹ Bostrom and colleagues evaluated rhBMP-2

combined with polylactic glycolic acid porous microspheres and autologous blood in healing of a 2-cm ulnar defects in New Zealand white rabbits. They found a similar dose-dependent relationship as the current study between the dose of rhBMP-2 and the mechanical strength of the healed defects. Additionally, the optimal rhBMP-2 dose (35–40 μ g rhBMP-2) for bone regeneration concurs between the two studies. Both studies indicate that at this optimal dose of rhBMP-2, bone strength returned to normal (intact limb) by 8 weeks.

Other studies evaluated the strength of demineralized bone matrix and rhBMP-induced bone within defects using collagenous carriers.^{40,41} Bolander and Balian found demineralized bone matrix induced active woven bone formation within large ulnar defects in rabbits, yet the strength of the newly formed bone was 50% that of intact bone after 12 weeks.⁴⁰ Cook et al.⁴¹ used rhOP-1 (rhBMP-7) with a collagen carrier to fill ulnar defects in rabbits. At 8 weeks post-operatively new bone had formed within the defect sites with primarily lamellar bone. Additionally, the torsional strength of the ulnae were comparable to the strength of the intact ulna after 12 weeks. The results of Cook et al.⁴¹ using rhBMP-7 with a collagen carrier are similar to those of Bostrom et al.³⁹ and the current study using rhBMP-2 with a polymer carrier. The local administration of rhBMP unequivocally improves bone healing of large defects compared to autograft, allograft, and demineralized bone matrix in laboratory animals. Additionally, the new bone formed within the defect achieves the mechanical strength of intact established bone.

In summary, this study showed that PDLLA provides a viable osteoconductive matrix for infiltration of new bone and a means to deliver rhBMP-2 to the defect site. It assembles a mechanically robust construct within 4 weeks of application within a rabbit radius CSD. The use of rhBMP with a polymer carrier provides a viable option for the repair of bony defects while eliminating immunogenic and donor site morbidity associated with standard graft materials (i.e., collagen, demineralized bone, autograft, and allograft). The robust mechanical strength demonstrated by the regenerated bone matrix produced in the rhBMP-2 + PLA composite makes this graft substitute a potential future option for many orthopedic procedures, including repair of large segmental bone defects as a result of trauma, neoplasia, or infection. It may also be applicable in prosthetic fixation, spinal arthrodesis, and fracture fixation.

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REFERENCES

- Goldberg, V. M.; Stevenson, S. Natural history of autografts and allografts. *Clin. Orthop. Rel. Res.* 225:7–16; 1987.
- Lane, J.; Sandhu, H. Current approaches to experimental bone grafting. *Orthop. Clin. N. Am.* 18:213–225; 1987.
- Glancy, G. L.; Brugioni, D. J.; Eilert, R. E.; Chang, F. M. Autograft versus allograft for benign lesions in children. *Clin. Orthop. Rel. Res.* 262:28–33; 1991.
- Laurie, S. W.; Kaban, L. B.; Mulliken, J. B.; Murray, J. E. Donor-site morbidity after harvesting rib and iliac bone. *Plast. Reconstr. Surg.* 73:933–938; 1984.
- Buck, B. E.; Malinin, T. I. Bone transplantation and human immunodeficiency virus. An estimate of risk of acquired immunodeficiency syndrome. *Clin. Orthop. Rel. Res.* 260:129–136; 1989.
- Horowitz, M. C.; Friedlaender, G. E. Induction of specific T-cell responsiveness to allogeneic bone. *J. Bone Joint Surg.* 73A:1157–1168; 1991.
- Tomford, W. Current concepts review: transmission of disease through transplantation of musculoskeletal allografts. *J. Bone Joint Surg.* 77A:1742–1754; 1995.
- Enneking, W. F.; Mindell, E. R. Observations on massive retrieved human allografts. *J. Bone Joint Surg.* 73A:1123–1142; 1991.
- Gross, A. E.; McKee, N. H.; Pritzker, K. P. H.; Langes, F. Reconstruction of skeletal deficits at the knee: a comprehensive osteochondral transplant program. *Clin. Orthop. Rel. Res.* 174:96–106; 1983.
- Bostman, O. M. Current concepts review, absorbable implants for the fixation of fractures. *J. Bone Joint Surg.* 73A:148–152; 1991.
- Constantino, P. D.; Friedman, C. D. Synthetic bone graft substitutes. *Otol. Clin. N. Am.* 27:1037–1074; 1994.
- Hench, L. L.; Andersson, O. H.; LaTorre, G. P. The kinetics of bioactive ceramics part III: surface reactions for bioactive glasses compared with an inactive glass. *Bioceramics* 4:156–162; 1991.
- Hollinger, J. O.; Schmitz, J. P.; Mixgala, J. W.; Hassler, C. An evaluation of two configurations of tricalcium phosphate for treating craniotomies. *J. Biomed. Mater. Res.* 23:17–29; 1989.
- Holmes, R. E.; Bucholz, R. W.; Mooney, V. Porous hydroxyapatite as a bone-graft substitute in metaphyseal defects. *J. Bone Joint Surg.* 68A:904–911; 1986.
- Klawitter, J.; Hulbert, S. Application of porous ceramics for the attachment of load bearing internal orthopedic applications. *J. Biomed. Res. Symp.* 2(1):161–229; 1971.
- Schwartz, Z.; Braun, G.; Kohavi, D.; et al. Effects of hydroxyapatite implants on primary mineralization during rat tibial healing: biochemical and morphometric analyses. *J. Biomed. Mater. Res.* 27:1029–1038; 1993.
- Heckman, J. D.; Boyan, B. D.; Aufdemorte, T. B.; Abbott, J. T. The use of bone morphogenetic protein in the treatment of non-union in a canine model. *J. Bone Joint Surg.* 73A:751–763; 1991.
- Marden, L. J.; Hollinger, J. O.; Chaudhari, A.; Turek, T.; Schaub, R. G.; Ron, E. Recombinant human bone morphogenetic protein-2 is superior to demineralized bone matrix in repairing craniotomy defects in rats. *J. Biomed. Mater. Res.* 28:1127–1138; 1994.
- Takaoka, K.; Nakahara, H.; Yoshikawa, H.; Mashuhara, K.; Tsuda, H.; Ono, K. Ectopic bone induction on and in porous hydroxyapatite combined with collagen and bone morphogenetic protein. *Clin. Orthop. Rel. Res.* 234:250–254; 1988.
- Urist, M. R. Bone: formation by autoinduction. *Science* 150:893–899; 1965.
- Cook, S.; Baffes, G.; Wolfe, M.; Sampath, T.; Rueger, D. Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone defect model. *Clin. Orthop. Rel. Res.* 301:302–312; 1994.
- Cook, S.; Wolfe, M.; Salkeld, S.; Rueger, D. Effect of recombinant human osteogenic protein-1 on healing of segmental defects in non-human primates. *J. Bone Joint Surg.* 77A:734–750; 1995.

23. Gerhart, T.; Kirker-Head, C.; Kriz, M.; Holtrop, M.; Henning, G.; Wang, E. Healing segmental femoral defects in sheep using recombinant human bone morphogenetic protein (rhBMP-2). *Clin. Orthop. Rel. Res.* 293:317–326; 1993.
24. Yasko, A. W.; Lane, J. M.; Fellingner, E. J.; Rosen, V.; Wozney, J. M.; Wang, E. A. The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). *J. Bone Joint Surg.* 74A:659–671; 1992.
25. Hollinger, J.; Jamiolkowski, D.; Shalaby, S. Bone repair and a unique class of biodegradable polymers: The poly(a-esters). Hollinger, J. O. ed. *Biomedical Applications of Synthetic Biodegradable Polymers*. Boca Raton, FL: CRC Press, Inc.; 1995:197–222.
26. Sandhu, H.; Kanim, L.; Kabo, J.; et al. Evaluation of rhBMP-2 with OPLA carrier in a canine posteriorlateral (transverse process) spinal fusion model. *Spine* 20:2669–2683; 1995.
27. Brekke, J. H. A rationale for delivery of osteoinductive proteins. *Tissue Eng.* 2:97–114; 1996.
28. Wozney, J.; Rosen, V.; Celeste, A.; et al. Novel regulators of bone formation: molecular clones and activities. *Science* 242:1528–1532; 1988.
29. Zegzula, H.; Buck, D.; Brekke, J.; Wozney, J.; Hollinger, J. Bone regeneration using recombinant human bone morphogenetic protein-2. *J. Bone Joint Surg.* 79A:1778–1790; 1997.
30. Burstein, A. H.; Frankel, V. H. A standard test for laboratory animal bone. *J. Biomech.* 4:155–158; 1971.
31. Levenston, M. E.; Beaupre, G. S.; Van der Meulen, M. C. H. Improved method for analysis of whole bone torsion tests. *J. Bone Min. Res.* 9:1459–1465; 1994.
32. Engesaeter, L. B.; Ekeland, A.; Langeland, N. Methods for testing the mechanical properties of the rat femur. *Acta Orthop. Scand.* 49:512–518; 1978.
33. Wozney, J. The bone morphogenetic protein family and osteogenesis. *Mol. Reprod. Dev.* 32:160–167; 1992.
34. Asselmeier, M.; Caspari, R.; Bottenfield, S. A review of allograft processing and sterilization techniques and their role in transmission of human immunodeficiency virus. *Am. J. Sports Med.* 21:170–175; 1993.
35. Nemzek, J.; Arnoczky, S.; Swenson, C. Retroviral transmission by the transplantation of connective tissue allograft. *J. Bone Joint Surg.* 76A:1036–1041; 1994.
36. Athanasiou, K.; Niederauer, G.; Agrawal, C. Sterilization, toxicity/biocompatibility and clinical applications of polylactic acid/polyglycolic acid copolymers. *Biomaterials* 17:93–102; 1996.
37. Ignatius, A.; Claes, L. *In vitro* biocompatibility of bioresorbable polymers: poly(L,DL-lactide) and poly(L-lactide-co-glycolide). *Biomaterials* 17:831–839; 1996.
38. Wintermantel, E.; Mayer, J.; Blum, J.; Eckert, K.; Luscher, P.; Mathey, M. Tissue engineering scaffolds using superstructures. *Biomaterials* 17:83–92; 1996.
39. Bostrom, M.; Lane, J.; Tomin, E.; et al. Use of bone morphogenetic protein-2 in the rabbit ulnar nonunion model. *Clin. Orthop. Rel. Res.* 327:272–280; 1996.
40. Bolander, M.; Balian, G. The use of demineralized bone matrix in the repair of segmental defects. Augmentation with extracted matrix proteins and a comparison with autologous grafts. *J. Bone Joint Surg.* 68A:1264–1274; 1986.
41. Cook, S.; Baffes, G.; Wolfe, M.; Sampath, T.; Rueger, D.; Whitecloud, T. The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. *J. Bone Joint Surg.* 76A:827–838; 1994.