Refinements in anesthetic and surgical techniques, implant design and application, and perioperative management have helped substantially improve treatment of complex fractures and other skeletal defects caused by trauma, disease, developmental deformity, and tumor resection. Nonetheless, an unfavorable wound environment caused by adverse tissue conditions, suboptimal surgical technique, or large body mass relative to fixation strength can lead to delayed healing or nonunion. Under these circumstances, some means of augmenting or accelerating bone regeneration would be desirable. A number of techniques have been used in attempts to achieve this goal, including various organic and inorganic osteoconductive and osteopromotive implants, biomechanical stimuli, electromagnetic stimuli, and numerous cellular and humoral factors. The gold standard for augmenting bone healing in humans and other animals, nonetheless, remains autogenous cancellous bone graft. More than 500,000 bone grafting procedures are performed annually in human patients in the United States, and 2.2 million are completed worldwide.¹ The number performed in companion animals, while undocumented, is also likely substantial.

Unfortunately, bone grafts have drawbacks. The additional anesthetic time or personnel needed for graft harvesting and the potential for an insufficient quantity of graft, limited access to donor sites, loss of osteogenic cells, donor site pain or hemorrhage, and failure of the donor bone are factors complicating cancellous autograft procedures. Similarly, allografts and xenografts carry the hazards of immune-mediated rejection and graft sequestration and, although unreported, the potential risk of disease transmission between donor and host. Bone banks are also costly to maintain. These factors have prompted the search for alternative bone augmentation technologies, and new cell therapy, gene therapy, and the use of bone growth and differentiation factors hold promise for clinical use. Although cell and gene therapies are not yet refined to the point of clinical application in orthopedic conditions, several recombinant BMPs are already being used clinically as alternatives or adjuncts to bone graft for treating a few well-defined clinical conditions in humans, possibly opening the way for their extralabel use in companion animals.

**Initial Discovery and Characterization of BMPs**

In 1965, Marshall Urist discovered that the extracellular matrix of allogeneic bone contained a previously unrecognized substance that elicited new bone formation when implanted extraskeletally (heterotopically) in rodents and rabbits.²,³ The sequence of events documented over a 4-week period was reminiscent of embryonic bone development and postnatal endochondral ossification. Undifferentiated mesenchymal cells migrated to the implantation site (chemotaxis) where they multiplied (mitosis) before differentiating into cartilage cells. A cartilaginous matrix was laid down and subsequently replaced with newly formed bone. The latter then remodeled to form an osseous structure possessing a central marrow core.⁴ The term BMP was introduced by Urist and Strates⁵ in 1971 to describe the substances responsible for these phenomena.

The osteoinductive properties of endogenous BMPs derived from the bones of numerous mammalian species have since been characterized.⁶ Use of endogenous human or bovine BMPs as adjuncts for the successful treatment of recalcitrant long bone nonunions and segmental defects in humans has also been reported.⁷,⁸ Widespread use of endogenous BMPs has not become commonplace, however, because of the lengthy purification process, small quantities of active substance yielded, and risk of disease transmission.

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>BMP</td>
<td>Bone morphogenetic protein</td>
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<tr>
<td>GDF</td>
<td>Growth differentiation factor</td>
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<tr>
<td>TGF-α</td>
<td>Transforming growth factor-α</td>
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<tr>
<td>rhBMP</td>
<td>Recombinant human bone morphogenetic protein</td>
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<tr>
<td>Dpp</td>
<td>Decapentaplegic</td>
</tr>
<tr>
<td>ACS</td>
<td>Absorbable collagen sponge</td>
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<tr>
<td>DBM</td>
<td>Demineralized bone matrix</td>
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<tr>
<td>POW</td>
<td>Postoperative week</td>
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<tr>
<td>RGD</td>
<td>Arginine-glycine-aspartic acid</td>
</tr>
<tr>
<td>HMSC</td>
<td>Human mesenchymal stem cell</td>
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<tr>
<td>CPC</td>
<td>Calcium orthophosphate</td>
</tr>
<tr>
<td>CRM</td>
<td>Compression resistant matrix</td>
</tr>
<tr>
<td>CESF</td>
<td>Circular external skeletal fixator</td>
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From the Orthopaedic Research Laboratory, Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA 01536. Dr. Kraus’ present address is the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Address correspondence to Dr. Kirker-Head.
In the mid 1980s, scientists developed the means of producing BMPs via recombinant DNA technology whereby the proteins were synthesized by bacteria (eg, *Escherichia coli*) or other cell lines (eg, Chinese hamster ovary cells) that had been transfected with a growth factor gene.11-13 This allowed synthesis of BMPs in comparatively large and pure quantities. As many as 20 BMPs and associated GDFs have since been identified, and all, with the exception of BMP-1, are members of the TGF-α signaling molecule superfamily.14,15 The proteins’ nomenclature can be confusing. Although endogenous BMPs are assigned the acronym BMP (eg, BMP-2 and -3), the recombinant human counterpart for each protein has the acronym rhBMP (eg, rhBMP-2 and -4). Furthermore, several BMPs have secondary names, and rhBMP-7, in particular, is preferentially referred to by many investigators as human osteogenic protein-1 (ie, hOP-1). Lastly, the corresponding DNA encoding each BMP is denoted by italicized lowercase letters (eg, bmp2 and bmp4).

In the postnatal skeleton, BMPs reside in periosteal cells, in mesenchymal cells of the marrow stroma, and along the collagen fibers of bone matrix. Bone morphogenetic proteins have also been isolated from osteosarcoma and chondrosarcoma cells.15 The temporal and spatial distribution of the BMPs during fracture healing is a complex, interactive, and site-specific process.16,17 Upon fracture, BMPs diffuse from resorbing bone matrix and activate osteo progenitor cells that, in turn, produce more BMPs.

The skeletal hallmark of the BMPs is their ability to enhance osteoinduction and bring about de novo bone formation.14 As chemotactic agents, they influence migration of progenitor and stem cells to the site of need. They then stimulate angiogenesis and stem cell proliferation, and as differentiating factors, they induce the maturation of stem cells into chondrocytes, osteoblasts, and osteocytes.15 When recombinant adenoviruses were used to express human BMP-2 through -15 singularly in vitro, all of the proteins with the exception of rhBMP-3 could elicit osteoinductive activity, implying osteogenic potential. Recombinant human BMP-2, -6, and -9 are additionally potent and induce differentiation of mesenchymal progenitor cells along an osteoblastic lineage. Bone morphogenetic protein-4 and -7 have an intermediate effect18 (Figure 1).

The fundamental importance of BMPs can be inferred from genetic, cellular, and biochemical data. Research indicates that BMPs have a pivotal role in regulating growth, differentiation, chemotaxis, and apoptosis of musculoskeletal cells.19-22 For example, the spatial distribution of the digital rays and interdigital spaces during limb bud development is controlled by BMPs through programmed cell death in the mesenchyme of the interdigital spaces.23,24 The proteins also help direct the development of numerous other organs and tissues, and they are pivotal in the establishment of the basic embryonic body plan.25,26,27

Additionally, these proteins have remained remarkably unchanged during the evolutionary process. For example, fruit flies (*Drosophila spp*) possess a BMP-like protein, dpp, which directs dorsal-ventral body patterning during early embryogenesis. It is sufficiently similar (75% homogeneity) to BMP-2 and BMP-4 that it can induce bone and cartilage when implanted subcutaneously in mammals.28 Going a step further, reef-building coral possess a dpp/BMP-2– and -4–like moiety that can cause phenotypic effects in *Drosophila* flies that mimic those of the dpp protein.29 This conservation of sequence homology across the ages implies the fundamental importance of these proteins. It has the additional benefit of allowing the BMPs of 1 mammalian species to be used in another without causing an adverse outcome.

**Molecular Characterization of the BMPs**

Bone morphogenetic proteins are synthesized as large precursor monomer peptides. Each has a terminal region that must be cleaved off before the preprotein can be secreted. The union of 2 preproteins then occurs, allowing formation of the active moiety.30-41 Heterodimers of BMP-2 and -7, for example, are more potent morphogens than are the corresponding homodimers.42 Once secreted from cells, BMPs may exert their actions locally, be bound by extracellular antagonists, or interact with extracellular matrix proteins that sequester the protein or enhance BMP activity by anchoring the protein to make it more available to target cells.43-46 In mammals, BMPs initiate their effects by binding with several cell surface receptors (type I and type II serine-threonine kinases).43 This leads to the downstream activation of intracellular transduction proteins (Smad 1, 4, 5, and 8), some of which move to the nucleus, where they regulate transcription of target genes. Within the cell, Smad proteins 6 and 7 inhibit BMP signaling,44 and extracellularly, other antagonists (eg, follistatin, noggin, and chordin) bind to BMPs, thereby preventing them from interacting with specific cell surface receptors.42,45

Within the context of endochondral ossification, which is the ubiquitous mechanism by which skeletal embryogenesis, postnatal bone development, and bone regeneration can occur, the BMPs collectively have a critical but variable role in effecting an appropriate outcome. For example, BMP-2 is essential for initiation of long-bone fracture healing, but more often, redundant systems allow the process to go to completion even if a particular BMP is deleted. For instance, the initiation of limb patterning and skeletal development can go forward in the absence of BMP-2 as long as BMP-4 remains present.46,49

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**Figure 1**—Illustration of the osteogenic hierarchy of BMPs. MSC = Mesenchymal stem cell. (Adapted from Cheng H, Jiang W, Phillips FM, et al. Osteogenic activity of the fourteen types of human bone morphogenetic proteins [BMPs]. J Bone Joint Surg Am 2002;84-A:1544–1552. Reprinted with permission.)
The complexities of the endochondral process at the molecular level continue to be defined. Most recently, the differential expression of BMP-related genes in specific regions of the growth plate has been reported. The BMP agonists, BMP-2 and -6, are expressed primarily in the hypertrophic zone, whereas BMP antagonists are expressed primarily in the resting zone. Collectively, these findings suggest there is a BMP signaling gradient across the growth plate that spatially controls chondrocyte proliferation and differentiation during chondrogenesis. Further evidence implies a complex interaction between this BMP system and another control system involving the Indian hedgehog protein and its antagonist, the parathyroid hormone-related protein. Conversely, BMPs may also regulate chondrocyte differentiation by mechanisms independent of both the Indian hedgehog and parathyroid hormone-related protein systems.

**Existing and Potential Clinical Applications of rhBMPs**

The development of recombinant BMPs set the stage for their commercialization. In North America, this effort was initially led by 2 biotechnology companies: Genetics Institute Inc (Boston, Mass), which developed rhBMP-2, and Creative BioMolecules Inc (Hopkinton, Mass), which developed rhBMP-7. The substantial financial investment required to fund the clinical development process, establish new market opportunities, and create a sales network subsequently prompted several large multinational corporations, including the health industry giants Wyeth, Stryker Biotech, Medtronic Sofamor Danek, and Yamanouchi Pharmaceuticals Co, to globally assume product development. These companies’ investment in developing BMPs for orthopedic applications is based on the proteins’ capacity to potentially accelerate bone healing and serve as an adjunct to or a replacement for traditional bone grafts.

Development of an optimal delivery mechanism for the proteins has been a primary obstacle to the clinical use of BMPs. Three principal strategies have been evaluated. In the first, the BMPs are systemically administered. The second technology uses gene transfer for delivery of selected BMP-synthesis genes to target cell populations, which, in turn, produce the selected BMPs at target sites. The third delivery strategy, which is presently used for existing clinical products, requires the BMPs to be locally delivered by implantation with a carrier matrix.

**Systemic administration**—Systemic administration is the least explored means of delivering BMPs to the body, principally because they are rapidly metabolized following injection, there is no effective oral means of administration, and the risk of unintended adverse effects following systemic administration cannot be ruled out. Nonetheless, various data imply the potential value of systemically administering BMPs. For example, serum concentrations of BMPs are high in growing children and humans with diseases such as Paget’s disease; conversely, serum concentrations can be low in patients with osteoporosis, and it is known that systemically administered rhBMP-2 reverses cartilage and bone loss in osteopenic mice. Conceivably, a carrier molecule capable of blocking rapid deactivation of BMP without hindering receptor activation may facilitate this mode of administration. Certainly, systemic administration of BMP has protective effects on several body systems. For example, rhBMP-7 reduces the severity of tissue damage in rats with induced colitis as well as those with cardiac ischemia-reperfusion injury. It also has a neuroprotective effect in rats with sustained cerebral hypoxia-ischemia, protects against renal damage, and may induce the repair of damaged renal tissue.

**Gene therapy**—Gene therapy involves delivery and transfer (transduction [eg, with a virus] or transfection [eg, with a plasmid]) of a gene sequence (transgene) to target cells, enabling them to synthesize the protein encoded by that gene. This treatment modality has several potential advantages over the use of recombinant proteins: growth factors may be delivered in a more biologically active form because the protein is synthesized in vivo; it may be possible to maintain sustained high concentrations of growth factors at the implantation site without adverse systemic effects; DNA is a stable molecule with a long shelf life; making storage straightforward; and the technology may be less expensive than using recombinant BMP systems.

A recent review of gene therapy-mediated delivery of BMPs has been published. Briefly, gene therapy can be systemic or local; genes can be introduced directly to the target site (in vivo technique), or selected cells can be harvested from the patient, expanded, genetically manipulated, and then reimplanted in the patient (ex vivo technique). Viral gene vectors (eg, adenovirus and retrovirus), generally modified to be replication deficient, and nonviral gene vectors (eg, plasmids, liposomes, molecular conjugates, and naked DNA) may be used to transfer exogenous DNA to the host cell nucleus, where it is incorporated into the host’s chromosomes or retained extrachromosomally (as an episome). Although nonviral vectors offer the potential advantages of large-quantity production, low immunogenicity, and no potential for reversion to a pathogenic form, there are a number of unresolved issues surrounding their use (eg, unpredictable gene expression and inflammatory response) and viral vectors are the preferred modality at this time.

Ex vivo gene transfer techniques have been used to encode BMPs in articular chondrocytes, peristeum, muscle, fibroblasts, and bone marrow cells. First-generation adenoviral constructs have been principally used. In vivo BMP viral vectors have also been tested and found to be effective at extraskeletal sites. Ultimately, healing at orthotopic sites is the goal, and successful healing of rabbit ulnar osteotomies in response to percutaneous injection of transgenic adenoviral–mediated delivery of BMP-6 has been reported. An increasing number of orthotopic models using spinal, craniofacial, and long-bone locations in several animal species have since been described.

A primary factor limiting the clinical usefulness of BMP gene therapy may be the immune response induced by the cellular or viral vector. However, in contrast to other applications, any resultant short-term transgene
expression may actually benefit BMP-transgene-derived bone formation because the comparatively transitory BMP presence may be sufficient to initiate the bone healing cascade and yet serve to limit excessive bone formation. Investigators are presently focusing on means to minimize any immune response, for example, with the use of so-called gutless adenoviral vectors from which all viral genes have been removed. Greater control of gene expression is also being sought, for example, through the use of inducible promoters that permit exogenous regulation of transgene expression by use of comparatively nontoxic, inert agents such as tetracycline. Others are exploring the use of multiple transgenes whose products, such as BMP-4 and parathyroid hormone, work synergistically to optimize the osteoinductive response.

**Carrier matrices**—Local delivery of BMPs in buffer solution will accelerate healing of fractures and bone defects. However, the osteogenic response is substantially less than that achieved when the proteins are combined with a carrier matrix. When radiolabeled rhBMP-2 and buffer were injected into critical-size osseous defects in the femoral neck of sheep, healing was better than that achieved with buffer alone but substantially less than that which occurred when rhBMP-2 was implanted with a bovine tendon–derived ACS carrier (Figure 2). This was attributed to the documented extended residence time at the implant site of the rhBMP-2–ACS combination. Although as little as 1 hour of exposure to BMP can prompt a cellular response, the carrier’s ability to delay the dispersion of the water-soluble and readily diffusible BMPs from the implant site enhances the proteins’ exposure to migrating stem cells and other growth and differentiation factors, likely prompting synergistic activity and a maximal osteogenic response. Matrices also serve as scaffolds for cell recruitment, attachment, proliferation, and differentiation. Some matrices, like calcium phosphate, have the added ability to attract and concentrate the host’s endogenous BMPs. Matrices might also protect BMPs from nonspecific lysis.

Matrices should be bioabsorbable so that they do not decrease the long-term biomechanical strength of the repair; should consist of a material that can be sterilized and is readily handled to facilitate intraoperative manipulation; should be biocompatible, nontoxic, cost-effective, and readily manufactured; and should have regulatory approval as a medical device. Also, injectable carriers must be sufficiently fluid to permit passage through hypodermic needles. The optimal choice of matrix will also be influenced by the anatomic location where the treatment is needed, the vitality of the soft tissue envelope, and the mechanical strain environment. Their importance warrants further description.

**Organic carriers**

Collagen formulations are organic carriers that have been extensively tested as BMP delivery vehicles. In the early years of BMP development, inactive DBM, largely type 1 collagen rendered devoid of endogenous morphogens, was used as a delivery vehicle when testing the osteoinductive potency of the BMPs. In an early study, long bone defect healing in large animals, an rhBMP-2–ovine inactive DBM composite was implanted into a critical-size, plate-stabilized, mid-femoral segmental defect in adult sheep. New bone was radiographically apparent at POW 3 or 4 and union at POW 12 (Figure 3). Defects left unfilled or implanted with inactive DBM alone produced negligible new bone. In a 1-year follow-up study, sheep femoral defects treated with the same rhBMP-2 composite exhibited a normal sequence of healing that resulted in formation of new cortices across the defect and recanalization of the adjacent medullary cavity. As many as 20 DBM allograft products, some containing endogenous BMPs, are now commercially available, principally as bone graft extenders. Demineralized bone matrix has seen little use as a BMP carrier, however, not least because the small particle size of such preparations slows osteogenesis, a cadaveric source is required, and the possibility of an immunogenic reaction or the transmission of disease exists.

In contrast, there is considerable enthusiasm for ACS carriers, largely because of their excellent safety record and their preexisting approval for use as hemostatic agents and wound coverings (thereby substantially reducing the cost of clinical development). Because BMP retention is a function of soaking time, the sponges are usually immersed in a BMP solution for up to an hour before implantation. The proteins differentially bind to the sponges, with rhBMP-2 and -6 having greater retention than rhBMP-4. Bovine type 1 tendon–derived ACS is presently the approved carrier for commercially available rhBMP-2 products. Marketed rhBMP-7 products also use collagen carriers; one is a particulate bovine bone–derived type 1 collagen matrix, and the other is a particulate collagen matrix.
combined with carboxymethylcellulose. The recent development of recombinant human collagen may provide for an even more reliable, predictable, and chemically pure carrier product, free of animal components and the risk of disease transmission.

Our laboratory is presently participating in a collaborative study to develop natural and recombinant silkworm silks as scaffolds for bone formation. Potential advantages of silk include the material’s strength, ability to resist failure in compression, stability at physiologic temperatures, and insolubility in aqueous and organic solvents. It is possible to engineer silk with a diverse range of surface chemistry configurations for modification and decoration. Initial studies reveal that natural silkworm silk films, made devoid of proinflammatory molecules (seracins) but modified by attaching an RGD peptide sequence to enhance stem cell adherence and spreading, are less inflammatory than either collagen or polylactic acid films. Successful healing of rat critical-size femoral defects with RGD-decorated silk scaffold and HMSCs predifferentiated along an osteoblastic lineage has been attained. Healing has also been achieved by use of silk scaffolds implanted with rhBMP-2 alone, rhBMP-2 and predifferentiated HMSCs, and rhBMP-2 and undifferentiated HMSCs (Figure 4).

Many synthetic organic polymers such as polyglycolic acid and poly(lactide-co-glycolide) have also been tested as BMP carrier matrices. Their major advantages are design flexibility (eg, fibers, sheets, and blocks) and the elimination of disease transmission risk.
be made bioabsorbable, and they are variably porous. With the same ovine femoral defect model described previously, successful healing at 1 year in 5 of 10 defects implanted with rhBMP-2 and poly(\(d,\ l\)-lactide-co-glycolide) was attained93 (Figure 5). Unfortunately, in 2 of the sheep in which the defect failed to heal, polymer particles were infiltrated locally by inflammatory cells.93 Excessive inflammatory responses, poor clearance, bulk degradation, excess swelling, rapid loss of mechanical strength, and local decreases in pH resulting from acid breakdown products preclude the clinical use of many synthetic organic polymers.87,94 However, novel polymers that break down by surface rather than by bulk erosion, providing a linear degradation profile, preservation of geometry, and intact surface as well as retention of mechanical strength, are undergoing evaluation.94 Injectable synthetic polymers possessing temperature-dependent, liquid-semisolid transitions, such that the carrier solidifies on cooling to body temperature, are also being assessed as percutaneous delivery vehicles for BMPs.95

By chemically cross-linking high–molecular-weight hyaluronic acid, biocompatible and biodegradable hydrogels have been formulated for the delivery of BMPs.96-98 Hydrogel-rhBMP-2 composite implants induce endochondral bone formation at heterotopic sites96 and healing of critical-size skull and long-bone defects in rabbits. Several formulations of hydrogel alone or in combination with other carriers have also been tested as injectable carriers for the BMPs.79 Most recently, synthetic polyethylene-glycol–based hydrogels decorated with RGD to promote cell adhesion and matrix metalloprotease cleavage sites to promote carrier degradation on cell infiltration have been successfully tested as a delivery vehicle for rhBMP-2 in a critical-size calvarial defect model in rats. Principal advantages of this system are its synthetic origin and its ability to advantageously direct local cell behavior and, in so doing, regulate rhBMP-2 release.100-102

**Inorganic carriers**

A variety of inorganic materials have been tested as carrier matrices for BMPs, including low- and high-temperature CPCs, calcium sulfate cements, bioactive glass, and metals.87,103 High-temperature CPCs (eg, hydroxyapatite and tricalcium phosphates) can be fabricated to mimic the 3-dimensional structure of trabecular bone, and some are partially load bearing. However, they have a prolonged residence time that can prolong healing. Additionally, they can only be surface-coated with BMP, which implies a short residence time for the protein.

In contrast, some low-temperature CPCs have the BMP incorporated into the cement, thereby extending the protein’s retention profile as it is gradually released during the resorption process.87 Most recently,
injectable calcium phosphate cements that set under endothermic conditions to form poorly crystalline hydroxyapatite have been a subject of interest. The endothermic setting eliminates thermal damage to the proteins, the calcium phosphate chemical structure enhances protein binding, and the poor crystallinity of the material enhances its resorption, thereby minimizing interference with bone healing. The composite accelerates healing of osteotomies in rabbits, dogs, and nonhuman primates. In all instances, acceleration of bone repair by up to 50% was observed. Accelerated healing of metaphyseal defects has also been reported in nonhuman primates. The principal advantages of an injectable product are the potential for percutaneous or minimally invasive delivery under fluoroscopic guidance and the ability to time administration for optimal effect, such as some time after initial open fracture repair when a greater number of responsive cells may be present and a more favorable microenvironment might exist as inflammation subsides.

**Composite matrices**

A large number of composite matrices have also been evaluated in the hope that the shortcomings of any particular carrier component can be offset by the strengths of another. For example, addition of biphasic calcium orthophosphate (15% hydroxyapatite and 85% tricalcium phosphate) granules to ACS and rhBMP-2 improved compression resistance of the implant and reduced the required dose of rhBMP-2 in a nonhuman primate model of lumbar intertransverse process arthrodesis. Interest has recently focused on another composite product that is an osteoconductive matrix composed of bovine type I collagen fibers (70%) coated with a porous hydroxyapatite (30%). When combined with rhBMP-14 (MP52; rhGDF-5) in nonhuman primate long-bone defect and spinal fusion models, the degree of bone formation achieved was equivalent to that obtained with an autograft. Preclinical human trials have recently been reported. The composite offers the benefits of being porous and having high wet strength, sponge-like resilience, radiolucency, osteoconductivity, and complete resorbability.

**Dose Response**

Regardless of application, the osteoinductive response to BMPs is generally dose dependent and inversely proportional to the species’ position on the phylogenetic scale. The quantity of rhBMP-2 or -7 needed clinically for osteoinduction is many times the amount of corresponding endogenous BMP derived locally or from implantation of autogenous bone graft. This likely reflects the autograft’s ability to provide a range of growth and differentiation factors as well as a respondent cell population at the implantation site, maximizing the opportunity for synergistic interaction, whereas the response to rhBMP must rely on locally preexistent cofactors, osteoblasts, and osteoprogenitor cells. Osteoinductive response is also predicated on the delivery vehicle, which frequently influences local BMP retention characteristics. It is clear that there is no single ideal pharmacokinetic profile that is predictive of success, and surgeons should note that more is not necessarily better. Because of the complex interaction between osteoblast and osteoclast signaling, higher-than-recommended concentrations of BMPs or rapid release from the carrier can result in transient osteoclastic bone resorption preceding bone formation, notably in metaphyseal trabecular bone. When rhBMP-2–ACS composites were implanted in an ovine femoral neck defect, transient bone resorption was apparent at margins of the defect before intraosseous bone formation healed the void. Clinically available rhBMP-2 is provided in 2.8-, 5.6-, and 8.0-mg formulations, whereas rhBMP-7 is provided in a 3.5-mg formulation.

**Specific Clinical Musculoskeletal Applications of BMPS**

**Fracture and nonunion repair**—Recombinant human BMP-2 delivered in a bovine tendon–derived ACS carrier is now approved in many countries as an adjunct for the treatment of acute tibial fractures in humans (Table 1). Recombinant human BMP-7 delivered in a type I bovine bone–derived collagen carrier is also approved in Europe, Australia, Canada, and the United States for the treatment of nonunions of adult human long bones (specifically the tibia in Europe). In a recent European clinical trial, the use of rhBMP-14 (rhGDF-5 or MP52) combined with an osteoconductive, mineralized collagen matrix not yet commercially available resulted in successful healing in 6 of 7 long-bone delayed unions or nonunions in humans. One patient was lost to follow-up.

Preclinical studies of rhBMP-2 and -7 in rabbits, dogs, goats, and nonhuman primates have been reported. Most recently, Faria et al. reported the treatment of external fixator–stabilized midshaft tibial osteotomies with a 1-mm gap in dogs. Seven dogs were untreated control animals, and 14 had the osteotomy sites wrapped with rhBMP-2 (0.05 or 0.2 mg/mL) delivered in an ACS. At POW 8, treatment with the higher dose of BMP resulted in faster radiographic healing, faster recovery of limb function, and closer-to-normal biomechanical properties.

The BMPs have been used less extensively in veterinary clinical patients. Paatsama et al. provided the first report when they described the use of partially purified endogenous canine BMP in coral and tricalcium phosphate carriers for the successful repair of a delayed union fracture and a pseudarthrosis in dogs. Subsequent reports documented the repair of a femoral14 and a radial15 nonunion treated with rhBMP-2 in dogs. Subsequently, the use of nonglycosylated BMP-2 in a fibrin matrix delivery vehicle was reported for the management of 8 long-bone atrophic nonunions in 5 cats and 3 dogs. An uncomplicated outcome was achieved in 6 cases, and in 2 of these, the implant was administered through a stab incision into the fracture gap without debridement. In a recent report, 4 dogs that had delayed union or nonunion of bone fractures, osteotomies, or arthrodeses were treated with either minimally invasive, fluoroscopically guided, percutaneous administration or with direct surgical application of rhBMP-2 (0.2 to 1.6 mg) by use of a calcium

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**Table 1**

<table>
<thead>
<tr>
<th>Fracture and nonunion repair</th>
<th>Recombinant human BMP-2 de - delivered in a bovine tendon–derived ACS carrier</th>
<th>Recombinant human BMP-7 de - livered in a type I bovine bone–derived collagen carrier</th>
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<td><strong>Fracture and nonunion repair</strong></td>
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<td><strong>Specific Clinical Musculoskeletal Applications of BMPS</strong></td>
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arthrodesis was performed. The humeroradial and humeroulnar articulations and olecranon fossa were debrided of articular cartilage and packed with CRM with 0.6 mg of rhBMP-2 and stabilized with compression bone plating. Fusion occurred rapidly, with robust callus observed by POW 11.

In another clinical case, one of the authors (RJB) used 0.4 mg of rhBMP-2 delivered locally in a synthetic apatitic calcium phosphate carrier, in combination with a plate and screws, to successfully treat a recurrent mid-diaphyseal femoral fracture in a 1.5-year-old German Shorthaired Pointer. The original fracture had been repaired 10 months earlier, but a torsional malunion developed and became infected. The original plate and screws had been removed 1 month previously, and the infection was treated on the basis of results of bacterial culture of intraoperative swab specimens. Refracture occurred at the original fracture location because less-than-ideal cortical remodeling was present. The torsional deformity was corrected at the time of restabilization. Healing with a robust amount of callus was evident by POW 6. At 1.3 years after revision, the fracture was solidly healed with ongoing recanalization of the medullary cavity (Figure 6).

Long-bone defects—Large long-bone defects are encountered less frequently in clinical veterinary patients than fractures; however, critical-size long-bone

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Table 1—Summary of the clinical availability of rhBMP products.

<table>
<thead>
<tr>
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<td>Medtronic Sofamor Danek, USA</td>
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<tr>
<td>Carrier matrix</td>
<td>i) Type I bovine collagen sponge and lumbar tapered fusion device or threaded fusion device</td>
<td>ii) Type I bovine collagen sponge and lumbar tapered fusion device</td>
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<td>Package size (US cost in dollars for the human clinical product)</td>
<td>i) 2.8 mL (4.2 mg), 8 mL (12 mg) or 5.6 mL (8.4 mg), or 8 mL (12 mg) of rhBMP-2</td>
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EU = European Union, CA = Canada, AU = Australia.
defects that lack the ability to spontaneously heal have been extensively used by researchers to evaluate BMPs’ osteoinductive capacity when delivered in a variety of modalities. Endogenous human BMP was used to supplement autogenous cancellous grafts in the successful healing of 6 traumatic segmental 3- to 17-cm-long tibial defects in humans. In 1999, 5 of 6 critical-size human fibular defects, created by osteotomy in the course of managing osteoarthritis of the knee, also healed successfully following implantation of rhBMP-7 with a type I collagen carrier.

In companion animal patients, endogenous partially purified moose BMP was used to fill the defects left following ulnar osteotomy in 2 dogs with incongruence and...
subluxation of the elbow joint. No other rhBMP-treated clinical defect studies have been reported, but future applications in the management of defects encountered following tumor resection or massive trauma are likely.

Vertebral fusion—Although endogenous and recombinant BMPs have been evaluated as a means of enhancing intervertebral fusion in numerous domestic animal species, all serving as models for the human disease, there is only 1 clinical report describing the protein’s use for this purpose in companion animals. An 8-month-old Border Collie with a severe congenital malformation and malalignment of the cervical portion of the vertebral column was successfully treated with an occipitocervical fusion performed dorsally by use of CRM, which spanned the entire dorsal cervical vertebrae, with 10.0 mg of rhBMP-2 in conjunction with locked plating stabilization. Robust bone formation was observed by POW 8. At POW 22, radiography revealed bridging of the cervical vertebrae with ongoing bone remodeling (Figure 7).

Vertebral arthrodesis is the most common indication for human autologous bone grafting, with as many as 250,000 procedures being performed each year in the United States. Of these, 5% to 45% end in nonunion. Accordingly, manufacturers have emphasized spinal fusion as a potentially useful application for BMPs, and several recombinant proteins are now approved for this purpose in humans (Table 1).

Craniomaxillofacial and mandibular indications—Numerous species including nonhuman primates have been used in craniomaxillofacial and mandibular studies characterizing the BMPs’ osteoinductive properties in the unique skeletal environment of the head where bones of endochondral and intramembranous origin lie in close proximity and also for their potential for clinical application. Furthermore, human craniofacial and mandibular defects as large as 13 × 11.5 cm have been successfully healed by use of endogenous BMP in combination with a variety of grafts and vascularized cutaneous flaps.

The clinical use of BMPs for management of cranio-maxillofacial or mandibular disorders in domestic species is, however, rarely reported. One of the present authors (RJB) used CRM with rhBMP-2 to successfully correct severe mandibular malocclusion in a 14-month-old Golden Retriever that was the result of a previous left hemimandibulectomy at 6 months of age for an atypical squamous cell carcinoma. After corrective right mandibular osteotomy, normal occlusion was reestablished and both mandibles were stabilized by plates spanning the bilateral defects (right defect, 1.5 cm; left defect, 7 cm). A fenestrated, monocortical rib graft was positioned beneath the left gingival surface to protect the rhBMP-2 composite from anticipated oral trauma as a result of chewing and was secured with a mini plate along the alveolar border; similarly, a mini plate was placed along the alveolar border of the right mandible. A mandibular reconstruction plate (right) and a locking mandibular reconstruction plate (left) were also secured to the ventrolateral borders of the mandibles. The mandibular gaps were filled with CRM with rhBMP-2 (left defect, 5.6 mg; right defect, 1.2 mg). New bone formation was identified by POW 12, and bony remodeling was evident at recheck examinations up to 4 years later. One author (RJB) has similarly treated 2 other mandibular lesions with partial mandibulectomy and immediate mandibular reconstruction. In 1 case (ossifying epulis) involving a 5-year-old mixed-breed dog, 0.8 mg of rhBMP-2 was delivered in a synthetic calcium phosphate carrier in combination with gap stabilization by use of a mini plate. In the other case (odontoma), the mandibular gap was filled by use of CRM with 2.0 mg of rhBMP-2 in combination with plate fixation (locking mini plate along the alveolar border and locking reconstruction plate along the ventrolateral mandible). New bone was identified within the gap by POW 8, and continued remodeling was present at recheck examinations up to 1.5 years later (Figure 8). Other potential craniomaxillofacial and mandibular applications for BMPs in veterinary clinical patients include management of head trauma and congenital or developmental abnormalities and treatment of large bone defects following excision of neoplasms.

Periodontal indications—Successful regeneration or healing of periodontal tissues in dentulous patients requires reattachment of connective and epithelial tissues to a completely avascular and almost impermeable tooth root surface. It also demands the coordinated integration of several embryologically different cellular phenotypes that contribute to the wound healing process, each following a predetermined maturation and anatomic allocation process. In edentulous human patients, augmentation of maxillary or mandibular alveolar bone is often an additional prerequisite to the placement of dental prostheses. A variety of tissue regeneration techniques evaluating the potential clinical use of BMPs for these purposes have undergone initial evaluation in animal models. A caprine model has been used to assess the feasibility of using an rhBMP-2–ACS composite to reinforce the boney floor of

Figure 7—Lateral radiographic view of the cervical vertebrae of a 13-month-old dog 5 months after performance of an occipitocervical fusion for a severe congenital malformation and malalignment with 2 locking plates and an rhBMP-2–CRM composite implant. Notice that the area is bridged dorsally from the skull to the last cervical vertebra, with the implant having abundant new bone formation.
the maxillary sinus prior to placement of a dental prosthesis. One sinus was implanted in each of 6 animals with the rhBMP-2–ACS composite and the contralateral sinus with the buffer–ACS composite as a negative control sinus. Radiography of sinuses implanted with the rhBMP-2–ACS composite revealed increasing radiopacity at the implant site throughout the postsurgical period, whereas control sinuses remained empty. At POW 12, substantial new bone formation was documented on the floor of the sinuses where the rhBMP-2–ACS composite had been implanted, whereas the control sinuses remained unchanged143 (Figure 9).

Both rhBMP-2 and -7 have subsequently been used to augment maxillary and mandibular bone prior to implantation of dental prostheses in humans. However, absorbable collagen sponge has proven to be a somewhat inadequate delivery matrix for this purpose, largely because of its failure to resist compression. Use of a more resilient xenogenic bone substitute mineral carrier may generate more substantial bone formation.144

Osseointegration of metallic implants—A titanium implant combined with rhBMP-2 and a collagen carrier already has regulatory approval for single-level anterior lumbar spinal fusion in humans in the United States and Europe, and other data imply that BMPs may enhance osseointegration of other metallic implants. Several studies145-147 in dogs have revealed the potential benefit of using recombinant BMPs for enhancing bone ingrowth around porous metallic implants during arthroplasty, but no clinical reports exist.

Distraction osteogenesis—Patients with large bone defects resulting from trauma or limb length discrepancies associated with congenital defects are often suitable candidates for distraction osteogenesis, in which viable osseous tissue is generated by the gradual separation of osteotomized bone margins. Several BMPs (BMP-2, -4, -5, -6, and -7) are active participants in the distraction osteogenesis remodeling process that occurs principally by intramembranous bone formation.125,148,149 A single injection of rhBMP-7 stimulated the rate of regenerative ossification and increased bone growth.149
mineral density during distraction osteogenesis in a femoral osteotomy model in rats, and a sequence of 3 injections of rhBMP-2 and buffer enhanced consolidation of regenerated bone in a tibial osteotomy model of distraction osteogenesis in sheep. However, no clinical reports document the use of recombinant BMPs with distraction osteogenesis in either companion animals or humans.

Clinical safety—Recombinant human BMP-2 and -7 have now been used in more than 10,000 human patients with generally favorable outcomes. However, their effects on the body are incompletely understood, and manufacturers and regulatory agencies continue to closely monitor patients for potential adverse events.

Potential Adverse Events

Excess bone formation—Recognizing that bone formation in response to rhBMP-2 and -7 is dose dependent and most prominent where a responsive cell population exists, such as on exposed bone surfaces, the careful placement of the BMP delivery vehicle and use of no more than the recommended dose of the protein are crucial. In many instances, this information has not been empirically derived, and it is likely that extralabel use of the proteins will require continued careful judgment by clinicians until such time that manufacturers can provide definitive recommendations for different clinical situations. Further, only the approved matrix should be used to avoid unwanted leaching of the BMPs beyond the implant site. Human spinal surgeons are particularly concerned about the potential for excess bone formation. The risk of BMP-induced bony overgrowth causing cord impingement or intertransverse fusion extending beyond the desired level exists, although data are inconclusive about how much of a risk this represents.

Malignancy—In vitro data suggest that rhBMP-2 may have an antiproliferative effect on human tumor
Pregnancy—Embryo and fetal development studies in animals treated with the rhBMPs are limited (rhBMP-2) or absent (rhBMP-7). Although administration of rhBMP-2 in gravid rats resulted in increased fetal weight and some skeletal variations, administration in gravid rabbits did not result in any abnormalities. Recognizing the organogenic actions of the BMP family and the potential for antibodies against BMP in treated patients, use in pregnant women is contraindicated. Although no evidence exists to imply a safety concern for pregnant veterinary patients, similar precautions might be appropriate.

Cost

The rhBMPs have been under development for clinical use since the late 1980s, but regulatory approval for their clinical application in humans in the United States was only granted in 2001 and 2002. Accordingly, pharmaceutical companies must now retrieve substantial research and development costs through market sales. Presently, the smallest available quantity of rhBMP-2 is a 4.2-mg (1.5 mg/mL) vial marketed at $3,600. Our own and others’ experience with dogs supports the clinical efficacy of 0.2 to 0.5 mg/mL applications of rhBMP-2, recognizing that larger lesions may require greater total volumes of the product. Accordingly, the human product can be diluted for use in dogs, thereby reducing the unit cost. Further, any unused liquid can be saved and refrozen at −70°C for later use. Recombinant human BMP-7 is only available in a 3.5-mg (1.4 mg/mL) quantity marketed at $5,000 for an implant and $6,000 for a putty.

Conclusions

A large body of data now documents the preclinical and clinical efficacy of recombinant BMPs for skeletal regeneration in humans and animals. Clinical use in veterinary patients is also being reported in peer-reviewed journals. Recombinant human BMP-2 and -7 have been approved by regulatory agencies for treatment of human clinical entities including acute long-bone fractures, recalcitrant long-bone nonunions, lumbar fusion, and oral maxillofacial and dental regenerative uses. Human clinical products have been made available for veterinary use. Many delivery modalities for the proteins have been investigated, and presently, collagen-based delivery matrices are used clinically. As more sophisticated delivery mechanisms such as percutaneous injection are developed, it is likely that the delivery matrices and range of clinical applications for the BMPs will expand substantially. Conceivably, the proteins could be used in conjunction with other growth and differentiation factors as well as with expanded responsive cell populations. The authors believe that BMPs represent an important addition to the veterinary surgeon’s armamentarium of products for the management of skeletal lesions.
References


