

# CURRENT CONCEPTS REVIEW

## THE ROLE OF GROWTH FACTORS IN THE REPAIR OF BONE

### BIOLOGY AND CLINICAL APPLICATIONS

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- ▶ Growth factors (bone morphogenetic protein, transforming growth factor-beta, fibroblast growth factor, platelet-derived growth factor, and insulin-like growth factor) are proteins secreted by cells that act on the appropriate target cell or cells to carry out a specific action.
- ▶ Because growth factors are expressed during different phases of fracture-healing, it has been thought that they may serve as potential therapeutic agents to enhance bone repair.
- ▶ The selection of an appropriate carrier or delivery system for a particular growth factor is essential in order to induce a specific biologic effect.
- ▶ There are a number of potential clinical applications for growth factors in the enhancement of bone repair, including acceleration of fracture-healing, treatment of established nonunions, enhancement of primary spinal fusion or treatment of established pseudarthrosis of the spine, and as one element of a comprehensive tissue-engineering strategy that could include gene therapy to treat large bone-loss problems.

Growth factors are proteins that serve as signaling agents for cells. They function as part of a vast cellular communications network that influences such critical functions as cell division, matrix synthesis, and tissue differentiation. The results of experimental studies have established that growth factors play an important role in bone and cartilage formation, fracture-healing, and the repair of other musculoskeletal tissues. Recently, with the advent of recombinant proteins, there has been considerable interest in the use of growth factors as therapeutic agents in the treatment of skeletal injuries. As growth factors become available as therapeutic agents, it is essential that orthopaedic surgeons understand their biological characteristics and clinical potential. The purpose of this review is to define the mechanisms of action, functions, and potential clinical applications of a variety of growth factors that may be used clinically to treat problems associated with the repair of bone.

#### **Growth Factors: General Concepts**

Growth factors are proteins secreted by cells that act on the appropriate target cell or cells to carry out a specific action. Three types of action are possible: (1) *autocrine*, in which the growth factor influences the cell of its origin or other cells identical in phenotype to that cell (e.g., a growth factor produced by an osteoblast influences the activity of another os-

teoblast), (2) *paracrine*, in which the growth factor influences an adjacent or neighboring cell that is different in phenotype from its cell of origin (e.g., a growth factor produced by an osteoblast stimulates differentiation of an undifferentiated cell), and (3) *endocrine*, in which the growth factor influences a cell that is different in phenotype from its cell of origin and located at a remote anatomical site (e.g., a growth factor produced by neural tissue in the central nervous system stimulates osteoblast activity). Thus, a growth factor may have effects on multiple cell types and may induce an array of cellular functions in a variety of tissues<sup>1,2</sup>.

Once a growth factor binds to a target cell receptor, it induces an intracellular signal transduction system that ultimately reaches the nucleus and produces a biological response. The binding of a growth factor to its receptor is known as a ligand-receptor interaction. These interactions are very specific and can range from simple, with a specific growth factor (ligand) binding to a single cellular receptor, to complex, with one or more ligands binding to one or more receptors in order to produce a ligand-receptor effect. Moreover, there is a redundancy in this biological system such that several forms of the same growth factor may bind to a single receptor or different growth factor receptors may be activated by a single ligand<sup>2,3</sup>.

Once the ligand-receptor interaction is established, the

receptor is activated by means of a change in its conformation. Receptors have both extracellular domains that bind to the ligand and intracellular domains that bind to and activate the signal transduction system. Part of this signal transduction system involves a so-called transcription factor, an intracellular protein that is activated as part of the signaling pathways initiated by the intracellular domain of a receptor. The activated transcription factor travels to the nucleus, binds to the nuclear DNA, and induces the expression of a new gene or set of genes (Fig. 1)<sup>4,5</sup>. It is the expression of these new genes by a cell that ultimately changes the characteristics of that cell. This sequence of events is similar to that which occurs with other agents such as steroid hormones, which bind to intracellular receptors and induce different types of intracellular signaling pathways<sup>3,4</sup>.

The type of activation as well as the specific transcription factor varies with the target cell, the growth factor-receptor combination, and the biological competency of the cell. For example, with growth factors in the transforming growth factor-beta (TGF- $\beta$ ) superfamily, signaling occurs through the activation of a transmembrane receptor complex formed by type-I and II serine/threonine kinase receptors<sup>2,3,5</sup>. This then leads to the so-called downstream activation of a group of transcription factors or intracellular signaling effectors called SMAD proteins. SMADs are a class of intracellular proteins that are involved in TGF- $\beta$  signaling. The term *SMAD* was created by merging the name of the *Caenorhabditis elegans* gene, *sma*, and the *Drosophila* gene, *MAD*. There are currently eight known members of this class of proteins<sup>5,6</sup>.

Bone morphogenetic protein (BMP) receptor binding and intracellular signal transduction follow the pathway outlined for TGF- $\beta$ , as BMPs are members of the TGF- $\beta$  superfamily. BMPs initially bind to the transmembrane type-II receptor with subsequent phosphorylation and activation of the type-I receptor. Two BMP type-I receptors (BMPR-1A and 2B) and one BMP type-II receptor have been identified. However, in contrast to the binding of TGF- $\beta$ , the BMP type-I receptor activates different SMADs within the cell, thus leading to a different cellular response<sup>5,7,8</sup>.

The biological activity of other growth factors is regulated by different receptor pathways. Fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) transduce signals to the cytoplasm via receptors that have tyrosine kinase activity. The major intracellular signaling pathways for these two growth factors have some overlap and include the Ras kinase cascades<sup>2</sup> (Table I).

Although the mechanisms for signal transduction by growth factors and their receptors have been delineated, there is a limited understanding of the ways in which these growth factors interact to regulate the repair of bone. There is general agreement that there must be so-called cross-talk between the various signaling pathways, but which cells and which growth factors are critical to this process remain to be determined. A better understanding of receptor activity and function will clearly be necessary in order to optimize the clinical use of these molecules<sup>2,9</sup>.

### Growth Factors and Skeletal Repair: Preclinical Studies

A number of growth factors have been shown to be expressed during different phases of experimental fracture-healing. On the basis of these findings, it is thought that these growth factors may serve as potential therapeutic agents to enhance the repair of bone. Among these growth factors are TGF- $\beta$ , BMP, FGF, PDGF, and insulin-like growth factor (IGF).

#### Transforming Growth Factor-Beta (TGF- $\beta$ )

TGF- $\beta$  belongs to a family of related proteins called the TGF- $\beta$  superfamily. This family of proteins includes the five isoforms of TGF- $\beta$  (TGF- $\beta$ 1 through TGF- $\beta$ 5), the BMPs, growth differentiation factors (GDFs), activins, inhibins, and Müllerian substance<sup>10-13</sup>. TGF- $\beta$  influences a broad range of cellular activities, including growth, differentiation, and extracellular matrix synthesis.

TGF- $\beta$  is found in many tissues, but it is particularly enriched in bone, platelets, and cartilage. It is presumed to be released by platelets after a clot is formed at the time of fracture<sup>14</sup>. It has been hypothesized that the release of TGF- $\beta$ 1 is associated with proliferation of periosteal tissue because there is positive immunostaining for TGF- $\beta$ 1 in the early fracture-healing period. However, the most intense staining occurs during cartilage cell proliferation and endochondral ossification<sup>11,15-17</sup>. Both chondrocytes<sup>15-17</sup> and osteoblasts<sup>14</sup> are enriched in receptors for TGF- $\beta$ , supporting the hypothesis that this family of growth

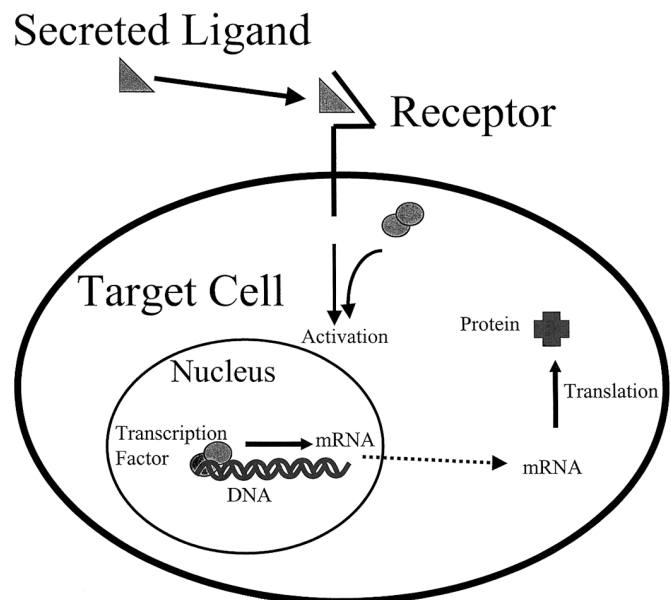


Fig. 1  
Diagram demonstrating the mechanism by which growth factors influence cell activity. The ligand binds to the extracellular domain of the receptor, and the intracellular domain of the receptor activates the signal-transduction system. A transcription factor, an intracellular protein, is activated as part of this process. The transcription factor migrates to the nucleus, binds to the nuclear DNA, and induces the expression of a new gene or set of genes.

TABLE I Growth Factors

Growth Factor	Source	Receptor Class	Function
Transforming growth factor beta (TGF- $\beta$ )	Platelets, bone extracellular matrix, cartilage matrix	Serine threonine sulfate	Pleiotropic growth factor stimulates undifferentiated mesenchymal cell proliferation
Bone morphogenetic protein (BMP)	Osteoprogenitor cells, osteoblasts, bone extracellular matrix	Serine threonine sulfate	Promotes differentiation of mesenchymal cells into chondrocytes and osteoblasts, promotes differentiation of osteoprogenitors into osteoblasts, influences skeletal pattern formation
Fibroblast growth factors (FGF)	Macrophages, mesenchymal cells, chondrocytes, osteoblasts	Tyrosine kinase	Mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
Insulin-like growth factors (IGF)	Bone matrix, osteoblasts, chondrocytes	Tyrosine kinase	Promotes proliferation and differentiation of osteoprogenitor cells
Platelet-derived growth factor (PDGF)	Platelets, osteoblasts	Tyrosine kinase	Mitogen for mesenchymal cells and osteoblasts; macrophage chemotaxis

factors affects the bone-healing process at all stages.

The role of TGF- $\beta$  in the repair of bone has been studied in experimental models involving subperiosteal injections in the femur<sup>17</sup> and calvaria<sup>18</sup>, critical-sized defects<sup>19-21</sup>, and bone ingrowth into prosthetic devices<sup>22</sup>. Joyce et al.<sup>17</sup>, using a subperiosteal injection model in the rat, demonstrated that injections of TGF- $\beta$ 1 could stimulate periosteal cells to undergo endochondral ossification. Lind et al.<sup>19</sup> analyzed the influence of continuous infusion of TGF- $\beta$ , delivered by means of a mini-pump, on plated unilateral mid-diaphyseal fractures of the tibia in thirty rabbits. The fractures were treated with either 1 or 10  $\mu$ g of TGF- $\beta$  per day for six weeks. The control group received injections of the delivery vehicle without growth factor. Bone-mineral content, the amount of callus formation, and bending strength were evaluated. There were no differences among the three groups with respect to bone-mineral content or cortical thickness. There was a significant increase in callus formation in both experimental groups compared with the control group ( $p = 0.01$ ). Mechanical testing with use of three-point bending demonstrated a significant increase in normal bending strength only when the group treated with 1  $\mu$ g of TGF- $\beta$  was compared with the control group ( $p = 0.03$ ).

Nielsen et al.<sup>20</sup> evaluated the efficacy of two different doses of TGF- $\beta$  (4 or 40 ng) injected every other day for forty days in a rat tibial fracture model. Mechanical testing showed a significant increase in ultimate load to failure (a measure of strength) in the group that had received the 40-ng dose compared with the group that had received the 4-ng dose and the control group (which had received no growth factor) ( $p < 0.01$ ). However, there were no differences with respect to stiffness or energy to failure between either of the two experimental groups and the control group.

Critchlow et al.<sup>21</sup> evaluated the effect of exogenous TGF- $\beta$ 2 on the healing of twenty-five rabbit tibial fractures under both stable and unstable mechanical conditions. In one group, the tibiae were fractured and then treated with a dynamic compression plate to achieve a stable mechanical system. In the other group, a 0.5-mm gap was produced between the ends of the fractured tibiae and the bones were fixed with a plastic plate to achieve an unstable mechanical system. The animals in both groups were treated with either 60 or 600 ng of TGF- $\beta$ 2. No mechanical testing was performed. In the animals with a stable mechanical construct that were treated with 600 ng of TGF- $\beta$ 2, there was abundant callus formation but no increase in bone content in the calluses. The 60-ng dose had a negligible effect on fracture-healing. In contrast, animals with an unstable mechanical construct had minimal bone and cartilage formation after treatment with either 60 or 600 ng of TGF- $\beta$ 2. These findings demonstrate that appropriate surgical management is required for healing and is essential in order for TGF- $\beta$ 2 to enhance skeletal repair.

It is difficult to draw conclusions regarding the efficacy of TGF- $\beta$  on the basis of these studies of experimental fracture-healing because different isoforms and doses of growth factor were used and different animal models were employed. Although the results of these studies confirm the hypothesis that TGF- $\beta$  enhances cellular proliferation, the osteoinductive potential of TGF- $\beta$  seems limited. The positive results in the studies by Lind et al.<sup>19</sup> and Nielsen et al.<sup>20</sup> seem to be attributable to the high doses of TGF- $\beta$  employed. The single injection regimen used in the study by Critchlow et al.<sup>21</sup> induced no increase in bone content, suggesting that the ability of TGF- $\beta$  to enhance bone repair may require frequent dosing or very high doses of the protein. Both of these requirements may not

be feasible in the clinical setting. Finally, since TGF- $\beta$  enhances cellular proliferation among a variety of cell types, there is some concern that it could lead to unforeseen side effects. Therefore, TGF- $\beta$  seems to have limited potential as an agent to enhance bone repair in the clinical setting.

### **Bone Morphogenetic Protein**

The BMPs are members of the TGF- $\beta$  superfamily, and thirteen individual molecules have been identified at this time<sup>6</sup>. Presently, BMP-2, 4, and 7 are known to play a critical role in bone-healing by means of their ability to stimulate differentiation of mesenchymal cells to an osteochondroblastic lineage. BMP-2, 4, and 7 use the same serine/threonine kinase receptor complex to initiate cell-signaling<sup>6</sup>.

The BMPs also play a critical role in cell growth and bone formation<sup>6</sup>. Mice deficient in BMP-2, 4, and 7 die either early during embryonic development or soon after birth. Mice deficient in BMP-2 have developmental abnormalities of the skull, hindlimb, and kidney<sup>6,23-25</sup>. Mice deficient in BMP-5 have short-ear deformities, and BMP-7 deficiency has been associated with hindlimb polydactyly and renal agenesis<sup>26-29</sup>.

The concept that there is a substance in bone that can induce new-bone formation was recognized by Marshall R. Urist in 1965 when he observed that a new ossicle had formed after the implantation of demineralized bone matrix in a muscle pouch in the rat<sup>30</sup>. He termed this phenomenon the *bone-induction principle* and later identified a protein responsible for this effect, which took on the name *bone morphogenetic protein*. More than twenty years later, in 1988, Wozney et al.<sup>31</sup> identified the genetic sequence of bone morphogenetic protein, which led to the identification of its various isoforms. With this genetic information, it is now possible to produce various BMPs with use of recombinant gene technology. These recombinant proteins will most likely form the basis for therapeutic applications involving growth factors in the immediate future.

A number of preclinical studies have assessed the efficacy of recombinant human BMPs (rhBMPs) in the healing of critical-sized bone defects and the acceleration of fracture-healing. Cook et al.<sup>32</sup> evaluated the effect of rhBMP-7 (also known as recombinant human osteogenic protein-1 or rhOP-1) on the healing of ulnar and tibial segmental bone defects in a study of twenty-eight African green monkeys. The ulnar defects, which were 2.0 cm long, were treated with 1000  $\mu$ g of rhOP-1 in 400 mg of bovine bone-collagen carrier. Control ulnar defects were treated with autogenous bone graft and bovine collagen carrier or with bovine collagen carrier alone. The tibial defects, which were also 2.0 cm long, were treated with 250, 500, 1000, or 2000  $\mu$ g of rhOP-1 in 400 mg of collagen carrier. Control tibial defects were treated with autogenous bone graft and bovine collagen carrier or with bovine collagen carrier alone. In two animals, the tibial defect was left untreated. The animals were killed at twenty weeks postoperatively. Healing of the defects was evaluated radiographically, histologically, and biomechanically. Radiographic evaluation revealed that five of the six ulnae and four of the five tibiae that had been treated with rhOP-1

healed by six to eight weeks. None of the six ulnae that had been treated with autogenous bone graft healed, but five of the six tibiae that had been treated with autogenous bone graft healed. None of the defects that had been treated with carrier alone or that had been left untreated demonstrated any signs of healing. Histological evaluation of defects that had been treated with rhOP-1 revealed the presence of new cortices, composed of both woven and lamellar bone, and normal-appearing marrow elements. Mechanical testing of the ulnae and tibiae that had been treated with rhOP-1 demonstrated an average torsional strength to failure of 92% and 69% of that of the contralateral, intact ulnae and tibiae, respectively. In contrast, the average torsional strength to failure of the tibiae that had been treated with autogenous bone graft was only 23% of that of the contralateral, intact tibiae. None of the ulnae that had been treated with autogenous bone demonstrated sufficient healing to undergo mechanical testing. These findings are consistent with those of another study in which Cook et al. evaluated the efficacy of rhOP-1 in the healing of critical-sized defects in a canine model<sup>33</sup>.

Recombinant human BMP-2 has also demonstrated efficacy in the healing of critical-sized defects in rat<sup>34</sup>, rabbit<sup>35</sup>, sheep<sup>36</sup>, and dog<sup>37</sup> models. Sciadini and Johnson<sup>37</sup> evaluated the efficacy of rhBMP-2, delivered in a collagen sponge, in the healing of a critical-sized radial defect that was stabilized with an external fixator in a dog model. Twenty-seven dogs underwent bilateral radial osteotomy with the creation of a 2.5-cm diaphyseal defect. All dogs were treated with either autogenous bone graft or a collagen implant containing 0, 150, 600, or 2400  $\mu$ g of rhBMP-2. The dogs were killed at twelve or twenty-four weeks after the operative procedure, and a complete radiographic, histological, and biomechanical analysis was performed. All defects that had been treated with either autogenous bone graft or with the various doses of rhBMP-2 showed union radiographically and histologically. None of the eight defects that had been treated with a collagen carrier alone healed. Of concern is that a dose-dependent occurrence of cyst-like bone voids was also noted. The biomechanical performance of the defects that had been treated with all three doses of rhBMP-2 was comparable with that of the defects that had been treated with autogenous bone graft and was significantly better than that of the defects that had been treated with the placebo ( $p < 0.0005$ ). However, the biomechanical performance of the defects that had been treated with the lowest dose of rhBMP (150  $\mu$ g) was superior to that of the defects that had been treated with the higher doses, and this finding was attributed to the lack of cyst-like voids. The specific mechanism by which these voids developed could not be determined, but the data suggest that the dose of rhBMP-2 protein may have to be adjusted for different clinical applications.

Bostrom and Camacho<sup>38</sup> evaluated the influence of rhBMP-2 on the healing of fresh fractures in a rabbit ulnar osteotomy model. Twenty ulnar fractures were treated with 200 mg of rhBMP-2, delivered in a type-I collagen sponge and applied as an onlay graft. Limbs that were treated with carrier alone or that were left untreated served as controls. Radio-

graphic evaluation and biomechanical testing were done at two, three, four, and six weeks after the operative procedure. BMP-2 accelerated the healing at the osteotomy site as assessed both radiographically and biomechanically. Between three and four weeks after the procedure, the limbs that had been treated with BMP-2 showed increased stiffness and strength compared with the untreated, intact ulnae. The untreated and collagen-carrier groups attained comparable values at four and six weeks after the procedure.

There has also been considerable interest in the use of an osteoinductive agent such as BMP to enhance active spinal fusion in order to avoid the operative morbidity associated with the harvesting of autogenous bone graft. The potential efficacy of rhBMP in the treatment of intertransverse process and posterior segmental spinal fusion has been evaluated in a variety of animal models. In those studies, rhBMP-2 was used in association with a variety of carriers, including collagen<sup>39</sup>, polylactic acid<sup>40</sup>, and copolymers (polylactic acid-polyglycolic acid)<sup>41</sup>. All of those investigations demonstrated successful fusion of the spine and, in most instances, the fusion mass at sites that had been treated with rhBMP-2 was greater than that at sites that had been treated with autogenous bone. However, the presence of voids in the fusion mass was noted in two studies in which either an open-cell polylactic acid polymer (OPLA) or a polylactic-polyglycolic acid carrier was used<sup>40,41</sup>. These voids did not impair the mechanical integrity of the fusion mass as demonstrated by biomechanical testing, but further study of the potential influence of the protein dose and a specific carrier on the formation of these voids is necessary.

Recombinant bone morphogenetic proteins have also been used in association with metallic cages to induce lumbar and cervical spinal fusions. Boden et al.<sup>42</sup> reported successful laparoscopic anterior spinal arthrodesis in five adult rhesus monkeys that had been treated with rhBMP-2 in a titanium interbody threaded cage. Two different doses of rhBMP-2 (0.75 and 1.5 mg/mL) were implanted in a collagen sponge and placed in the cage. A solid fusion of the lumbar spine was achieved in association with both doses of the recombinant protein. In contrast, a solid fusion was not achieved in two animals that had been treated with a collagen sponge only.

Although the results of these preclinical studies have been promising, the relatively high doses of rhBMP required to induce adequate bone formation suggest that large amounts of recombinant protein may be required to produce a clinically important effect. This raises serious concerns regarding safety and cost<sup>6,38</sup>. Moreover, in order to exert their biological activity, the recombinant BMPs must be delivered via carriers. The carriers that have been tested most frequently for rhBMP include collagen matrix<sup>33,34,36-38</sup>, demineralized bone matrix<sup>33,39</sup>, and synthetic polymers<sup>36</sup>. It is unclear if these carriers are the best vehicles for presenting these molecules to receptors or responding cells. Development of more effective ways of exposing responding cells and tissues to bone morphogenetic proteins will likely be needed in order to maximize the clinical efficacy of these factors.

### *Fibroblast Growth Factors*

The fibroblast growth factors (FGFs) are a family of nine structurally related polypeptides that are characterized by their affinity for the glycosaminoglycan heparin-binding sites on cells and are known to play a critical role in angiogenesis and mesenchymal cell mitogenesis<sup>43-45</sup>. The most abundant FGFs in normal adult tissue are acidic fibroblast growth factor (FGF-1 or  $\alpha$ -FGF) and basic fibroblast growth factor (FGF-2 or  $\beta$ -FGF). Both FGF-1 and FGF-2 promote growth and differentiation of a variety of cells, including epithelial cells, myocytes, osteoblasts, and chondrocytes. The mitogenic effects of FGF-1 have been associated with chondrocyte proliferation<sup>46,47</sup>, while FGF-2 is expressed by osteoblasts and is generally more potent than FGF-1<sup>48</sup>.

The FGF family of peptides transduces signals via a group of four receptors that contain distinct membrane-spanning tyrosine kinases<sup>49,50</sup>. Mutations in these FGF receptors have been associated with abnormalities in endochondral ossification and intramembranous ossification. For example, mutations in fibroblast growth factor receptor-3 (FGFR-3) have been linked to several skeletal dysplasias, including achondroplasia, thanatophoric dysplasia (lethal neonatal dysplasia), and hypochondroplasia (a mild form of achondroplasia). These three dysplasias are the results of dominant missense mutations of the FGFR-3 gene. Achondroplasia is caused by a single amino acid change (arginine to glycine) in the transmembrane portion of the cell-surface receptor<sup>51,52</sup>.

Both FGF-1 and FGF-2 activity have been identified during the early stages of fracture-healing. Since these factors are associated with angiogenesis and chondrocyte and osteoblast activation, there has been interest in their ability to enhance skeletal repair<sup>53</sup>. Kato et al.<sup>54</sup> evaluated the effect of a single local injection of recombinant human fibroblast growth factor-2 (rhFGF-2) on the healing of segmental tibial defects in rabbits. A 3-mm bone defect was stabilized with an external fixator, and various doses (0, 50, 100, 200, and 400  $\mu$ g) of rhFGF-2 were injected. Healing was assessed with plain radiographs, histological analysis, and an evaluation of bone-mineral content with use of dual energy x-ray absorptiometry. A dose-dependent effect on healing, bone volume, and the mineral content of new bone was noted, with significant effects at concentrations of  $\geq 100$   $\mu$ g ( $p < 0.01$ ). Treatment with 100  $\mu$ g of FGF-2 increased the volume and bone-mineral content by 95% and 36%, respectively, compared with controls. Kato et al. concluded that a single injection of FGF-2 could enhance bone formation.

Nakamura et al.<sup>55</sup> assessed the effect of rhFGF-2 on the healing of tibial fractures in forty-one beagle dogs. A transverse osteotomy was created, and the tibia was stabilized with an intramedullary nail. Either 200  $\mu$ g of rhFGF-2 or vehicle alone was injected into the fracture site. The animals were killed at two, four, eight, sixteen, and thirty-two weeks after the fracture, and the fracture sites were assessed with regard to callus formation, morphological characteristics, and strength. By two weeks after the fracture, the rhFGF-2 group demonstrated an increase in the number of periosteal mesenchymal cells as well as increased differentiation of those cells into chondrocytes and os-

teoblasts. In addition, intramembranous ossification was more pronounced in the rhFGF-2 group. The rhFGF-2 group had an increase in the area of callus formation at four weeks and an increase in bone-mineral content at eight weeks. A maximal increase in the osteoclast index (the number of osteoclasts divided by the callus perimeter) was noted in the rhFGF-2 group at four weeks, while similar findings were noted in the control group at eight and sixteen weeks. In the rhFGF-2 group, reduction in callus volume began at eight weeks and fracture strength showed recovery at sixteen weeks. In contrast, callus volume in the control group did not change significantly from eight to sixteen weeks and fracture strength was low at sixteen weeks. Maximum load, bending stress, and energy absorption were significantly greater in the rhFGF-2 group than in the control group at both sixteen ( $p < 0.05$ ) and thirty-two weeks ( $p < 0.05$ ), even though fracture-healing had occurred in both groups. These results suggest that rhFGF-2 accelerates bone repair and also stimulates remodeling of the callus, a process that restores the biomechanical properties to the bone.

The ability of rhFGF-2 to accelerate fracture-healing in a higher species was confirmed in a nonhuman primate fracture model<sup>56</sup>. In that study, rhFGF-2 and hyaluronic acid were combined into a viscous gel formulation that was percutaneously injected into a 1-mm non-critical-sized osteotomy defect in the fibulae of baboons. An osteotomy in the contralateral fibula was left untreated to serve as a negative control. Intact fibulae from an additional group of necropsy animals served as positive controls. The osteotomy sites were treated with three different doses of rhFGF-2. The sites that had been treated with rhFGF-2 had a larger callus, greater bone volume, and increased osteoblastic activity. There were significant differences between energy to failure ( $p \leq 0.01$ ) and load at failure ( $p \leq 0.05$ ) between the treated and untreated osteotomy sites. No differences in torsional stiffness were observed when treated animals were compared with untreated controls. A dose response was not found, which suggests that a threshold amount of rhFGF-2 in this formulation will enhance the bone-repair process but a higher dose will not improve healing. The results of these studies<sup>54-56</sup> suggest that FGF may have the most potential as an adjunctive agent to enhance clinical skeletal repair.

#### **Growth Hormone and Insulin-Like Growth Factors**

Growth hormone and insulin-like growth factors (IGFs) play critical roles in skeletal development. Growth hormone is currently used clinically to treat patients with short stature<sup>57</sup>. In addition, because of its systemic effects there is interest in the use of growth hormone to treat osteoporosis and to enhance fracture-healing. Growth hormone participates in the regulation of skeletal growth<sup>9,58</sup>. It is released by the anterior lobe of the pituitary gland in response to stimulation by growth hormone-releasing hormone (GHRH), a hormone secreted by the hypothalamus. It then travels through the circulation to the growth plate and the liver, where target cells are stimulated to release IGF<sup>57,58</sup>. As both growth hormone and IGF are actively involved in skeletal development, their role in the repair and remodeling

of the adult skeleton have become a topic of interest. Two IGFs have been identified: IGF-1 and IGF-2. Although IGF-2 is the most abundant growth factor in bone, IGF-1 has been found to be more potent and has been localized in healing fractures in rats and humans<sup>59,60</sup>. Therefore, studies evaluating the role of IGFs in fracture-healing have concentrated on IGF-1.

A number of studies have been performed in different animal models with use of different doses and methods of administration to assess the influences of growth hormone and IGF on skeletal repair. The results have varied, and therefore it is difficult to determine the potential role of either growth hormone or IGF in the enhancement of fracture-healing. Bak et al.<sup>60</sup> assessed the effect of four doses of biosynthetic human growth hormone (0.08, 0.4, 2.0, and 10.0 mg/kg/day) on fracture-healing in ninety Wistar rats. Animals received either no injection or twice-daily injections of growth hormone or saline solution (control group) beginning seven days before the fracture and continuing until the animals were killed at forty days after the fracture. Biomechanical testing demonstrated increased ultimate load to failure, stiffness, and energy absorption in association with the 2.0 and 10.0-mg doses of growth hormone. An increase in the ultimate stress to failure was only seen in association with the 10.0-mg dose.

Carpenter et al.<sup>61</sup>, in a unilateral tibial osteotomy model in rabbits, found that intramuscular injections of human growth hormone did not have a significant effect on normal fracture-healing. The osteotomy sites in twenty-seven rabbits were stabilized with an external fixator, and each animal received an injection of either recombinant human growth hormone (150 µg/kg) or saline solution five times per week. The rabbits were killed at four, six, and eight weeks after the operation, and the tibiae were evaluated with a four-point bending test. In addition, the serum levels of IGF-1 were serially evaluated to determine the systemic response to the intramuscular injection of human growth hormone. There were no significant differences between the experimental and control groups with regard to the weekly radiographic findings. In addition, although the rabbits treated with growth hormone had higher serum levels of IGF-1 than the untreated controls did, there was no relationship between the serum level of IGF-1 and the results of the biomechanical tests.

The role of IGF-1 in stimulating intramembranous bone formation was studied in a calvarial defect model in rats<sup>62</sup>. Experimental animals were subjected to continuous systemic administration of IGF-1 for fourteen days via a subcutaneous osmotic pump, whereas control animals were treated with saline solution alone. The calvarial defects that had been treated with 2 mg of IGF-1 for two weeks healed via intramembranous ossification. The results of that study suggest that IGF-1 may have a role in enhancing bone formation in defects that heal via intramembranous ossification. However, the role of IGF-1 as an agent to enhance fracture-healing or spinal fusion requires further study.

#### **Platelet-Derived Growth Factor (PDGF)**

PDGF is secreted by platelets during the early phases of frac-



ture-healing and has been identified at fracture sites in both mice<sup>9</sup> and humans<sup>63</sup>. In vitro studies have demonstrated PDGF to be mitogenic for osteoblasts<sup>64</sup>. However, the role of PDGF in fracture-healing and bone repair has not been clearly defined.

Nash et al.<sup>65</sup> evaluated the efficacy of PDGF in the healing of unilateral tibial osteotomies in seven rabbits. Each osteotomy site was treated with either 80 µg of PDGF in a collagen sponge or with a collagen sponge alone. The animals were killed after twenty-eight days. Radiographic analysis at two and four weeks demonstrated an increase in callus density and volume in the animals that had been treated with PDGF compared with the controls. Histological analysis demonstrated a more advanced state of osteogenic differentiation both endosteally and periosteally in the animals that had been treated with PDGF than in the controls. A three-point bending test revealed no differences in strength between the tibiae that had been treated with PDGF and the intact, contralateral tibiae. Although the histological findings suggested that PDGF has a beneficial effect on fracture-healing, only a small number of animals were analyzed and the mechanical testing data were equivocal. Moreover, the small size of the study does not support robust statistical criteria. At the present time, the therapeutic role of PDGF in fracture-healing remains unclear.

### Carriers and Delivery Systems for Growth Factors

The ability to deliver a molecule so that it will induce a specific biologic effect is critical to the success of growth factor therapy. The success of the delivery system may depend on the anatomic location where the treatment is needed, the vitality of the soft-tissue envelope, and the mechanical strain environment provided by the fixation or reconstructive system. The kinetics of release of the growth factor from its delivery system may vary depending on the chemistry of the factor or the delivery system and the influence of the host environment. For these reasons, certain conditions must be considered when selecting an appropriate carrier or delivery system: (1) the ability of the system to deliver the growth factor at the appropriate time and in the proper dose, (2) the presence of a substratum that will enhance cell recruitment and attachment and will potentiate chemotaxis, (3) the presence of a void space to allow for cell migration and to promote angiogenesis, and (4) the ability of the delivery system to biodegrade without generating an immune or inflammatory response and without producing toxic waste products that would inhibit the repair process<sup>66</sup>.

A number of carrier and delivery systems, including type-I collagen<sup>34,35</sup>, synthetic polymers<sup>36</sup>, and hyaluronic acid gels<sup>53,56</sup>, have been used to deliver recombinant proteins in experimental and clinical models. A variety of so-called bone-graft substitutes, including demineralized bone matrix, calcium phosphate-containing preparations (such as hydroxyapatite, coralline hydroxyapatite, and  $\alpha$ -BSM [ETEX, Cambridge, Massachusetts]), and Bioglass<sup>67-69</sup>, are also potential carriers for recombinant proteins.

In clinical trials in humans, type-I collagen has been used

as a carrier for BMP, in conjunction with metal cages, to induce fusion in the spine<sup>70</sup>. This protein has been considered an attractive carrier because of its fibrillar structure and the fact that it is the most abundant protein in the extracellular matrix of bone. It also promotes mineral deposition and can bind noncollagenous matrix proteins that also initiate mineralization<sup>6</sup>. In addition, collagen has already been cleared for marketing by the United States Food and Drug Administration for several clinical applications, suggesting that it has a favorable safety profile and a proven efficacy in specific applications. While there are some concerns regarding the use of allogeneic collagen with respect to its potential to induce an immune response, abundant data suggest that this risk is low<sup>32,33,37</sup>.

Although collagen has been used successfully as a carrier for BMP in a variety of animal models, large doses of BMP have been required to produce an osteogenic effect in clinical trials of spine fusion<sup>70</sup> and periodontal applications<sup>71,72</sup> in humans. This has raised the concern that collagen interferes with the pharmacokinetics of the release of BMP and in some way limits the resultant osteogenic response. The pharmacokinetic profile of rhBMP-2 was evaluated with use of an assay in which the protein was implanted in a muscle pouch with use of a variety of carrier systems (including a type-I collagen sponge, tricalcium phosphate, hydroxyapatite, and demineralized bone matrix)<sup>73,74</sup>. The typical pharmacokinetic profile of BMP-2 release consisted of an initial burst effect with a half-life of less than ten minutes. This effect was carrier-dependent. A carrier-dependent secondary release with a half-life of between one and ten days was then noted. The collagen sponge lost 30% of the recombinant protein in the initial burst phase, followed by continuous release with a half-life of three to five days. This pharmacokinetic profile paralleled the degradation of the collagen sponge. In contrast, mineral-based delivery systems showed the same initial burst release profile but in the secondary phase there was diminished release because a substantial fraction of the protein was bound irreversibly to the mineral particles<sup>73,74</sup>.

Demineralized bone matrix preparations are particularly attractive as potential carriers for growth factors because they are osteoconductive and may have some osteoinductive potential as well. To our knowledge, these preparations have not been tested in combination with recombinant proteins in humans. In addition, Johnson et al.<sup>75</sup> demonstrated that purified BMP and demineralized bone enhance bone formation at nonunion sites in humans.

Polymers have also received much attention as potential delivery vehicles. Both polylactic acid (PLA) and polyglycolic acid (PGA), for example, are used as suture materials and, because of their biocompatibility profile and ability to bind protein, it is natural to consider using them as scaffolds to deliver peptide molecules. However, further investigation of the degradation profiles of various polymers is necessary to ensure that they degrade in a manner that does not stimulate an inflammatory response. In addition, it will be necessary to enhance the bonding of these materials to either host bone or soft tissue<sup>76,77</sup>. Strategies will need to be developed to create a

biomechanically stable construct between these carriers and the host bone and/or surrounding soft tissue.

Retention of the recombinant protein at the implantation site for a sufficient period to promote progenitor cell migration and cell proliferation has been shown to enhance osteoinductive activity. The osteoinductive potential of thermoreversible biomaterials containing BMP-2 that can be injected into an anatomic site is currently under investigation. These polymers are in a liquid phase at room temperature and then harden at physiologic temperatures in the body<sup>78</sup>.

Bioglass<sup>68,69</sup> and calcium phosphate-based materials such as hydroxyapatite<sup>77,79-82</sup>, coralline hydroxyapatite<sup>83-87</sup>, and tricalcium phosphate<sup>85,88-90</sup> have been shown to be biocompatible and to provide osteoconductive scaffolds that potentially could be combined with growth factors to enhance bone repair<sup>67,68,91-93</sup>. The disadvantages of these materials include poor handling characteristics and concerns about overall bioresorbability and limited potential for remodeling and an unclear understanding of their effects on bone strength<sup>91</sup>. Recently, there has been substantial interest in  $\alpha$ -BSM as a carrier for recombinant proteins. This poorly crystalline calcium phosphate apatite has several potential advantages as a carrier: (1) its crystalline structure simulates the mineral phase of bone and enhances remodeling into host bone, (2) it can be hydrated in saline solution to form a paste with excellent handling characteristics, and (3) since the paste hardens in the body via an endothermic reaction, degradation of proteins or antibiotics incorporated into the cement should not occur<sup>67</sup>. Studies are currently in progress to investigate the utility of  $\alpha$ -BSM as a clinically effective carrier for BMPs.

Recently, hyaluronic acid has been used as a carrier for mesenchymal stem cells and as a delivery vehicle for FGF-2<sup>53,56,94</sup>. A normal constituent of the extracellular matrix of articular cartilage and soft connective tissues, hyaluronic acid has also been shown to facilitate fetal development by enhancing cell migration and tissue morphogenesis. It has been suggested that growth factor composites with hyaluronic acid and derivatives of this molecule will support cell growth in a variety of clinical applications<sup>95</sup>. Solchaga et al.<sup>94</sup> tested the ability of a hyaluronic acid-based carrier to bind rabbit mesenchymal progenitor cells and enhance osteogenic differentiation in an in vivo assay. Culture-expanded bone-marrow-derived mesenchymal progenitor cells were placed on either a porous calcium phosphate ceramic carrier vehicle or two different hyaluronic acid sponges with different pore sizes and degradation profiles. The composites were then implanted subcutaneously into nude mice. Standard light and scanning electron microscopy were used to determine the ability of the implants to bind and retain mesenchymal progenitor cells and to support chondrogenesis and osteogenesis. In general, the hyaluronic acid sponges were superior to the calcium phosphate ceramic carrier with respect to the numbers of cells loaded per unit volume of the implant. The hyaluronic acid sponges, which had a longer time to degradation, were also superior to the ceramic with respect to the amount of cartilage and bone that formed in their pores.

As noted above, a hyaluronic acid-based gel was used as a carrier for FGF-2 in a nonhuman primate fracture model<sup>56</sup>. A single direct injection of the FGF-2 hyaluronic acid formulation enhanced local fracture-healing. Histological analysis revealed that osteotomy sites that had been treated with this growth factor composite had enhanced periosteal reaction, vascularity, and cellularity when compared with the untreated controls. There was no evidence of an inflammatory response to the hyaluronic acid gel. However, as no control group received just the hyaluronic acid gel, it is difficult to determine the specific role of the growth factor in enhancing fracture-healing.

While it is likely that there is no ideal carrier or delivery system for all growth factors or biological therapies, it is still unclear whether any of the currently known carriers have been truly optimized for clinical applications. This field of study, which is as important as the study of the growth factor molecules, cells, and genes themselves, will require much more emphasis as the field of biologic intervention in clinical therapeutics progresses.

#### *Gene Therapy as a Method of Growth Factor Delivery*

Although several recombinant proteins may soon be available as therapeutic growth factors for specific clinical applications, there is concern that a single dose of exogenous protein will not induce an adequate biologic response in patients, particularly in situations in which the viability of the host bone and surrounding soft tissues is compromised. To address this potential concern, a better strategy for protein delivery may be gene therapy. Gene therapy involves the transfer of genetic information to cells. When a gene is properly transferred to a target cell, the cell synthesizes the protein encoded by the gene<sup>96</sup>. Therefore, with gene therapy, the genetic message is delivered to a particular cell, which then synthesizes the protein. In general, the duration of protein synthesis after gene therapy depends on the techniques used to deliver the gene to the cell. Both short-term and long-term expression are possible. Chronic diseases, such as osteoporosis or rheumatoid arthritis, for example, would probably require long-term expression. However, the treatment of most bone-repair problems may only require short-term protein production<sup>97</sup>.

Several gene therapy options are currently under investigation. First, gene therapy can be applied either regionally or systemically. Second, the gene can be introduced directly to a specific anatomic site with use of an in vivo technique or it can be introduced via an ex vivo approach in which cells are harvested from the patient, the DNA is transferred to these cells in tissue culture, and the genetically modified cells are then administered back to the patient<sup>97</sup>.

An important aspect of gene therapy is the application of appropriate vectors for genes. Vectors are agents that enhance the entry and expression of DNA in a target cell. They may be of viral or nonviral origin. Viruses are efficient vectors because the delivery and expression of DNA is a critical aspect of their normal life cycle. When a virus is used as a vector, essential portions of its genome must be deleted to render it replication-deficient and to create space in its genome for the insertion of



the therapeutic DNA. Insertion of therapeutic DNA in exchange for a portion of the viral genome, which would otherwise confer upon the virus the ability to replicate, is accomplished by a process known as *homologous recombination*. The process that involves the transfer of functional genetic information from the recombinant vector (virus) into the target cell is known as *transduction*. This is accomplished when the virus that contains the therapeutic DNA binds to the cell, usually via a receptor-mediated process, and then enters that cell. The DNA then enters the nucleus of the cell, where it may become integrated into the host genome or may remain extrachromosomal. It is then possible for the transduced cell to produce and secrete the growth factor encoded by the DNA<sup>98-100</sup>.

A major concern related to the use of viral vectors is the subsequent recombination of the defective virus with viruses in the host cell, resulting in the generation of replication-competent viruses with the ability to multiply in the patient. In addition, cells infected with certain viruses (e.g., adenoviruses) produce not only the transgene product but also other viral proteins. These viral proteins may elicit an immune response in the host, which can limit the duration of protein expression by the transduced cells<sup>95-97</sup>. Both viral and nonviral vectors have been used to heal critical-sized defects and to induce fusion in the spine in both rabbits and rats (Fig. 2)<sup>66,101-105</sup>.

### Clinical Applications

There is a great deal of interest in the development of clinical applications for growth factors in the enhancement of bone repair, including (1) acceleration of fracture-healing (particularly in patients who are at high risk for nonunion), (2) treatment of established nonunions, (3) enhancement of primary spinal fusion, (4) treatment of established pseudarthrosis of the spine, and (5) as one component of a comprehensive tissue-engineering strategy that could include gene therapy to treat large bone-loss problems.

#### Fracture-Healing

Approximately 5% to 10% of fractures sustained in the United States are associated with delayed healing or nonunion<sup>106</sup>. Impaired fracture-healing is associated with a number of risk factors, including poor blood supply, associated soft-tissue injury, extensive bone loss, instability, infection, poor general medical condition, and smoking. Traditionally, problems related to fracture-healing have been treated with operative intervention, which often involves the use of an autogenous bone graft. However, bone graft-harvesting procedures are associated with a morbidity rate of 10% to 30%, and only limited amounts of autogenous bone are available<sup>107</sup>. Therefore, alternative strategies designed to enhance the healing of acute fractures and to improve the treatment of delayed unions and nonunions are required. Three biologically based strategies have shown promise as new technologies to enhance fracture repair: use of exogenous growth factors, mesenchymal stem cell therapy, and gene therapy.

Current evidence suggests that among the factors that have been investigated to date, BMPs appear to have the most

osteoinductive potential. Clinical trials have been performed to assess the efficacy of recombinant proteins in the treatment of fibular defects<sup>108</sup> and tibial nonunions<sup>109</sup> as well as for spinal arthrodesis<sup>70</sup> in humans. A prospective, randomized, double-blind study was performed to assess the efficacy of using OP-1 (BMP-7), delivered in a type-I collagen carrier, for the treatment of a critical-sized (approximately 15-mm) fibular defect in twenty-four patients who were undergoing high tibial osteotomy<sup>108</sup>. The fibular defects were treated with either 2.5 mg of recombinant OP-1 and a type-I collagen carrier, demineralized bone matrix alone, or type-I collagen alone. Five of the six patients treated with OP-1 demonstrated new bone from six weeks onward. Bridging of the defect was noted in five of these six patients at ten weeks. Four of the six patients treated with demineralized bone matrix had bridging of the defect at ten weeks. None of the defects treated with collagen alone healed.

The efficacy of recombinant OP-1 was also assessed in a prospective, randomized, partially blinded clinical trial involving 122 patients with 124 tibial nonunions<sup>109</sup>. Treatment consisted of intramedullary nail fixation and implantation of either recombinant rhOP-1 in a type-I collagen carrier or autogenous iliac bone graft. Nine months following the operative procedure (the primary end point of the study), 81% (fifty-one) of the sixty-three nonunions that had been treated with rhOP-1 and 85% (fifty-two) of the sixty-one nonunions that had been treated with autograft were judged to have been treated successfully according to clinical criteria ( $p = 0.524$ ). In that study, a clinical success was defined as full weight-bearing with less-than-severe pain at the fracture site. At nine months, radiographic analysis revealed that 75% of the nonunions that had been treated with rhOP-1 and 84% of those that had been treated with autograft had united ( $p = 0.218$ ). Therefore, there was no significant difference with respect to either clinical or radiographic outcome between the patients who had been treated with recombinant rhOP-1 and those who had been treated with autograft (Fig. 3). The Food and Drug Administration recently granted a Humanitarian Device Exemption for the use of the OP-1 device to treat recalcitrant nonunions of long bones (nonunions that have failed to respond to other treatment modalities).

The available preclinical data on the efficacy of TGF- $\beta$ , IGF, and PDGF in the treatment of nonunion or delayed union are insufficient to make predictions regarding the future clinical utility of these factors. These factors may have potential if used in combination with each other or with other growth factors, but the regulatory and licensing issues inherent in the development of combination therapies may be complex. PDGF is currently available for the enhancement of nonosseous wound-healing<sup>110</sup>. Its efficacy in this application may provide insights into its potential application for the treatment of skeletal wounds and defects.

#### Spinal Fusion

Spinal fusion is one of the most commonly performed operations in orthopaedic surgery, with more than 983,000 such pro-

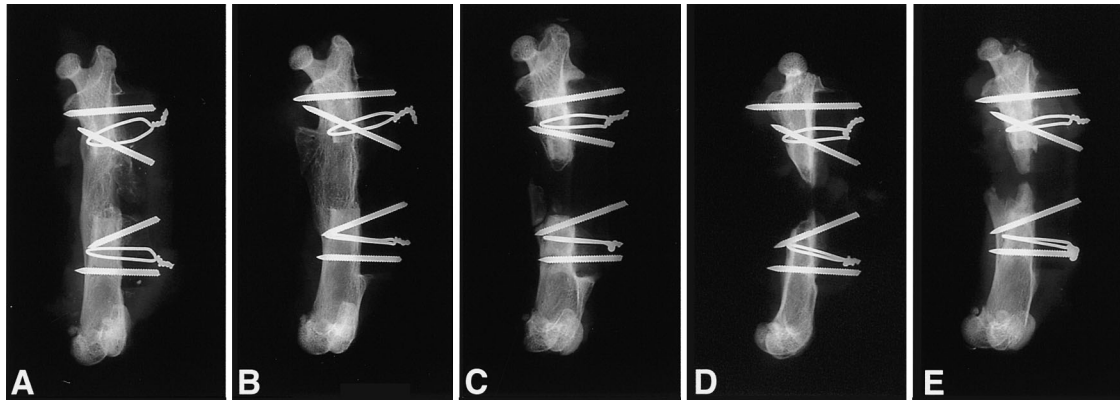


Fig. 2

Radiographs, made two months postoperatively, demonstrating differences in the healing of critical-sized femoral defects in rats that had been treated with (A) BMP-2-producing bone-marrow cells created via adenoviral gene transfer, (B) 20 µg of rhBMP-2, (C) β-galactosidase-producing bone-marrow cells (cells infected with an adenovirus containing lacZ gene), (D) noninfected rat-bone-marrow cells, or (E) guanidine-extracted demineralized bone matrix alone. Dense trabecular bone formed within the defects that had been treated with the BMP-2-producing cells, and the bone remodeled to form a new cortex. The defects that had been treated with rhBMP-2 healed but were filled with lace-like trabecular bone. Minimal bone repair was noted in the other three groups. (Reprinted from: Lieberman JR, Daluiski A, Stevenson S, Wu L, McAllister P, Lee YP, Kabo JM, Finerman GAM, Berk AJ, Witte ON. The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats. *J Bone Joint Surg Am.* 1999;81:905-17.)

cedures performed each year<sup>11</sup>. Approximately 33% (327,000) of these procedures involve bone-grafting. However, while autogenous bone-grafting is generally a successful method for enhancing spinal fusion, nonunion rates of 5% to 35% have been reported<sup>139,41,70</sup>. A number of factors, including the mechanical instability of the spine and its fixation, the quality of the bone and bone mass, the health of the surrounding soft tissue, the

type of bone graft used, and the concurrent use of medications and drugs such as nicotine, affect bone-graft incorporation and the success of spinal fusion.

A pilot study in humans demonstrated that recombinant human BMP-2 can be used to induce spinal fusion<sup>70</sup>. In a multicenter randomized trial, fourteen patients underwent a single-level anterior interbody fusion of the fifth lumbar and

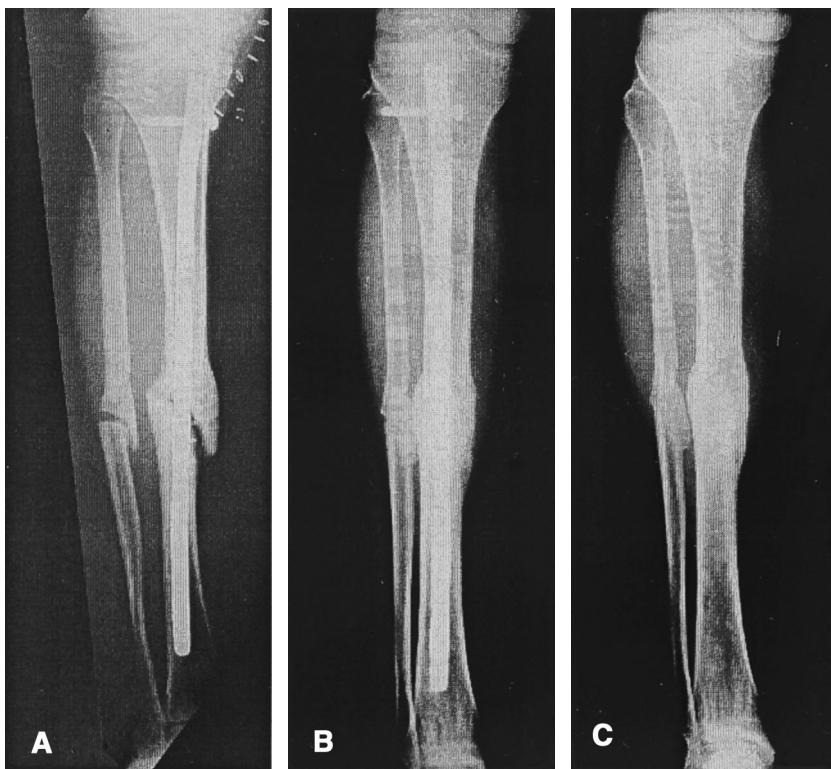


Fig. 3

Radiographs of a thirty-four-year-old man who was treated with osteogenic protein 1 (OP-1) after having sustained a closed comminuted tibial fracture in a motor-vehicle accident. Prior treatment had included intramedullary rod fixation, insertion of fresh bone autograft, a fibulectomy, and external electrical stimulation. The clinical and radiographic results were considered successful at both nine and twenty-four months after treatment with an intramedullary rod and OP-1. A, Immediate postoperative radiograph. B, Radiograph made nine months after treatment with OP-1. C, Radiograph made twenty-four months after treatment with OP-1. (Reprinted from: Friedlaender GE, Perry CR, Cole JD, Cook SD, Cierny G, Muschler GF, Zych GA, Calhoun JH, LaForte AJ, Yin S. Osteogenic protein-1 [bone morphogenetic protein-7] in the treatment of tibial nonunions. A prospective, randomized clinical trial comparing rhOP-1 with fresh bone autograft. *J Bone Joint Surg Am.* 2001;83-A Suppl 1[Pt 2]: S151-8).

first sacral vertebrae with use of a tapered titanium fusion cage. Eleven patients were treated with a cage filled with 10 mg of rh-BMP-2 in a collagen carrier, and three control patients were treated with a cage filled with autogenous bone graft. Six months after the procedure, all eleven patients who had been treated with recombinant protein and two of the three patients who had been treated with autogenous graft had evidence of fusion on plain radiographs and computed tomographic scans. No neurologic, vascular, or systemic complications were reported. Although the data appear promising, more patients will need to be studied in order to confirm the efficacy and safety of this method. In addition, the administration of a large dose (10 mg) of recombinant BMP may be costly, again suggesting that a collagen carrier may not be the most efficient method for BMP delivery. The role of other growth factors in enhancing spinal fusion requires further analysis.

The results of both preclinical and human studies suggest that growth factors may have an important role in spinal fusion procedures. The ability to deliver growth factors either as a protein or via gene therapy may lead to the development of less invasive operative techniques, such as laparoscopic spinal fusion. Such a development carries the potential for reducing operative morbidity, shortening time to wound-healing, and diminishing costs.

There has been some frustration associated with the amount of time that it has taken for growth factors used in the treatment of bone repair problems to become available to surgeons and their patients. Although OP-1 is now available on a restricted basis and approval for the use of rhBMP to enhance spinal fusion appears imminent, the regulatory approval process remains arduous. While all efforts to ensure product safety for this young and otherwise healthy group of patients are of paramount importance, the efficacy of growth factors in the enhancement of bone repair is not easy to demonstrate. The process of normal fracture-healing is already biologically optimized, and it is often difficult to simulate the human biological environment in an animal. Growth factors may be degraded more quickly in humans than in animals, the biology of the receptor-ligand interactions may differ, and the pharmacokinetics of the activity of growth factors may be less favorable in humans. Finally, although there are many settings

in which orthopaedic surgeons might want to use a growth factor to enhance skeletal healing, the assessment of healing in a scientifically sound and quantitative way is difficult. For example, valid imaging techniques have not yet been developed to determine if certain types of fractures are healed, to demonstrate the extent of the bone repair that occurs after bone-grafting of osteolytic lesions associated with revision total joint arthroplasty, or to determine if fusion actually occurs when a metallic cage has been placed in the spine.

The clinical application of growth factors has the potential to greatly improve the treatment of conditions requiring bone repair. The development of appropriate delivery systems should enable surgeons to initiate successful tissue-engineering strategies and to develop minimally invasive surgical techniques that can reduce both morbidity and costs. Carefully designed clinical trials will be needed to test the efficacy of these strategies. Enhancing our understanding of the critical interplay between growth factor biology and the properties of the host environment will guide the applications of genetic engineering in orthopaedic treatments.

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In support of their research or preparation of this manuscript, one or more of the authors received grants or outside funding from National Institutes of Health. In addition, one or more of the authors received payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity (Genetics Institute, Stryker Biotech, Orquest, Musculoskeletal Transplant Foundation). Also, a commercial entity (Genetics Institute, Stryker Biotech, Musculoskeletal Transplant Foundation) paid or directed, or agreed to pay or direct, benefits to a research fund, foundation, educational institution, or other charitable or nonprofit organization with which the authors are affiliated or associated.

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