

## Simple Carrier Matrix Modifications Can Enhance Delivery of Recombinant Human Bone Morphogenetic Protein-2 for Posterolateral Spine Fusion

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**Study Design.** A nonhuman primate lumbar intertransverse process arthrodesis model was used to evaluate modifications to a plain collagen sponge to deliver recombinant human bone morphogenetic protein-2 (rhBMP-2).

**Objectives.** To evaluate the feasibility of enhancing the delivery of rhBMP-2 with the established collagen sponge carrier by adding biphasic ceramic phosphate (BCP) granules (15% hydroxyapatite, 85% tricalcium phosphate) or allograft chips to provide compression resistance for posterolateral spine arthrodesis.

**Summary of Background Data.** Recombinant human bone morphogenetic protein-2 was successfully delivered with a resorbable collagen sponge in a rabbit intertransverse process fusion model. Success in nonhuman primates required a higher dose (6–9 mg) of rhBMP-2 and a more compression-resistant matrix (ceramic) than plain collagen. The limitation of the ceramic carrier was its radiopacity, which made radiographic detection of new bone formation difficult.

**Methods.** Nine adult rhesus monkeys underwent bilateral posterolateral intertransverse process arthrodesis at L4-L5. The animals were divided into three groups (n = 3 each) based on the graft material implanted: 1) autogenous iliac crest bone (5 cm<sup>3</sup>/side); 2) collagen sponge and 15:85 BCP granules loaded with rhBMP-2 (3 mg/side); and, 3) collagen sponge and allograft chips loaded with rhBMP-2 (3 mg/side). The monkeys were killed 24 weeks after surgery. Inspection, manual palpation, radiography, computed tomographic scans, and histology were used to assess fusion.

**Results.** All six monkeys with rhBMP-2 delivered in the collagen/15:85 BCP carrier and the collagen/allograft chips carrier achieved solid spine fusions, whereas only one of three animals fused with autogenous bone graft. Histologic analysis of the bone induced by rhBMP-2 showed normal trabecular bone and bone marrow elements.

**Conclusions.** The addition of either 15:85 BCP granules or allograft bone chips to the existing resorbable collagen-

sponge matrix enhanced delivery of rhBMP-2 in the posterolateral spine. The combination matrices were more compression resistant and had improved radiographic resorption properties that permitted easy radiographic visualization of new bone. In addition, a lower dose of rhBMP-2 (3 mg/side) was successful compared with the dose previously used with the plain collagen sponge (6 mg/side). [Key words: bone graft substitute, ceramic, hydroxyapatite, nonhuman primate, recombinant human bone morphogenetic protein-2, spinal fusion, tricalcium phosphate, allograft] **Spine 2003;28:429–434**

A posterolateral intertransverse process fusion is the most common type of fusion performed in the lumbar spine. A successful fusion using autogenous iliac crest bone graft occurs in less than 90% of instrumented cases, and non-union, or failed fusion, may occur in up to 45% of uninstrumented cases in some reports.<sup>8–10,12,13,15,16,18,21</sup> Autogenous bone is considered the most successful bone graft material used for spinal arthrodesis and is presently the “gold standard” against which all other graft materials are compared. Complications with its use may occur in as many as 15% to 25% of patients.<sup>1,2,14</sup> Graft harvest complications include increased blood loss and risks from transfusion; increased surgical morbidity from an additional operative site for graft harvest, including chronic donor site pain; increased operative time; and additional cost. The quantity of bone available to harvest may be insufficient for long, multisegment arthrodesis or in patients with previous graft harvests.

The limitations of the autogenous bone graft have prompted the investigation of a variety of bone graft substitutes and graft extenders.<sup>3</sup> Osteoinductive growth factors, including bone morphogenetic proteins (BMPs), have also been investigated.<sup>26,28</sup> Earlier studies in rabbits and dogs demonstrated that lumbar intertransverse process spine fusions could be produced by recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered in a collagen sponge or open-cell polylactic acid carrier matrix.<sup>23–25</sup> Subsequently, the authors have shown that bone induction in primates is more difficult than in lower animals and requires a higher dose (up to 32 mg/side) of rhBMP-2 with the plain collagen sponge carrier and a barrier to prevent compression of the sponge by surrounding soft tissue.<sup>5,17</sup> Earlier studies in the nonhuman primate also demonstrated that a moderate dose (6–9 mg) of rhBMP-2 could result in bone induction in the posterolateral spine area

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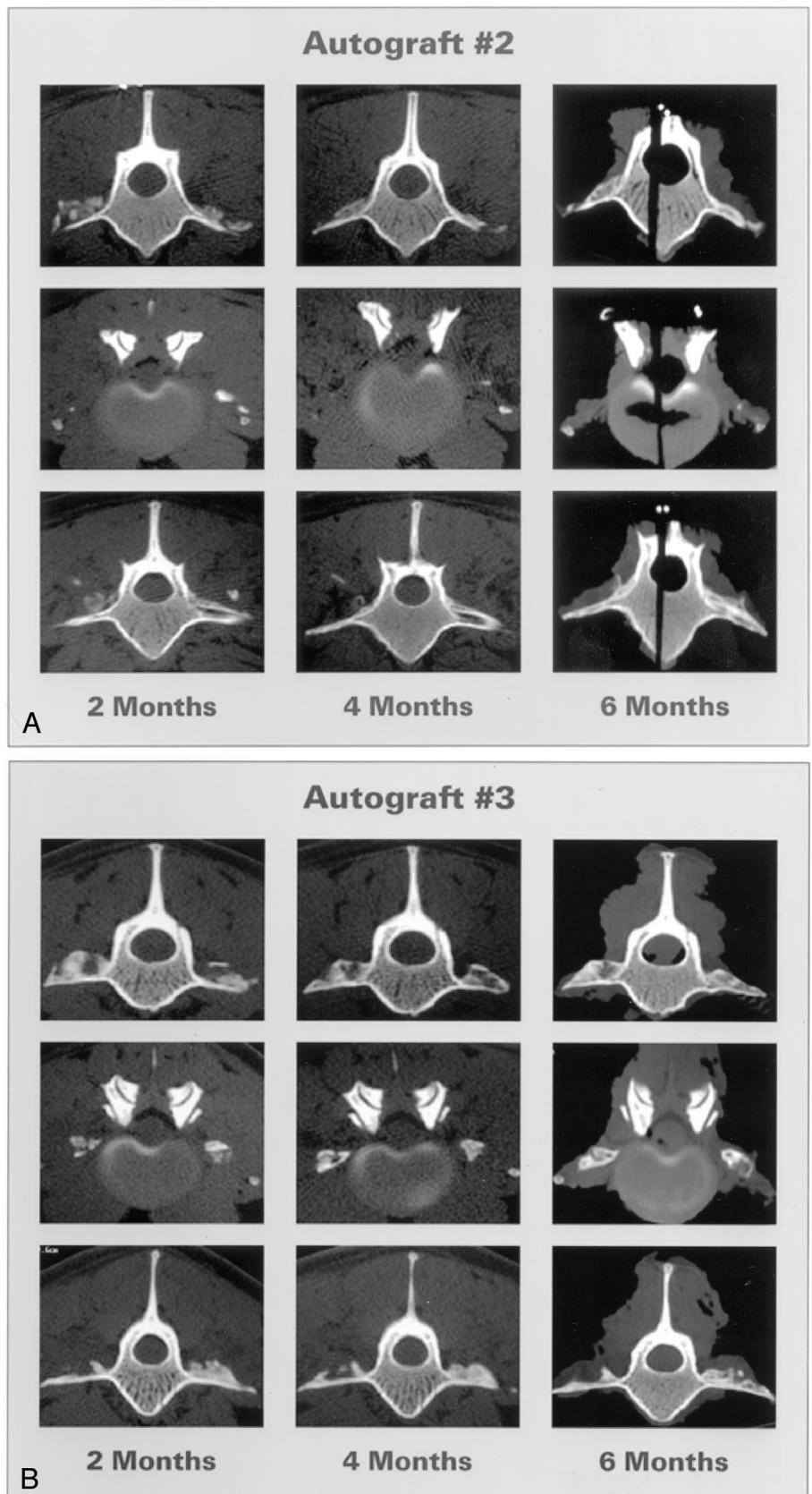


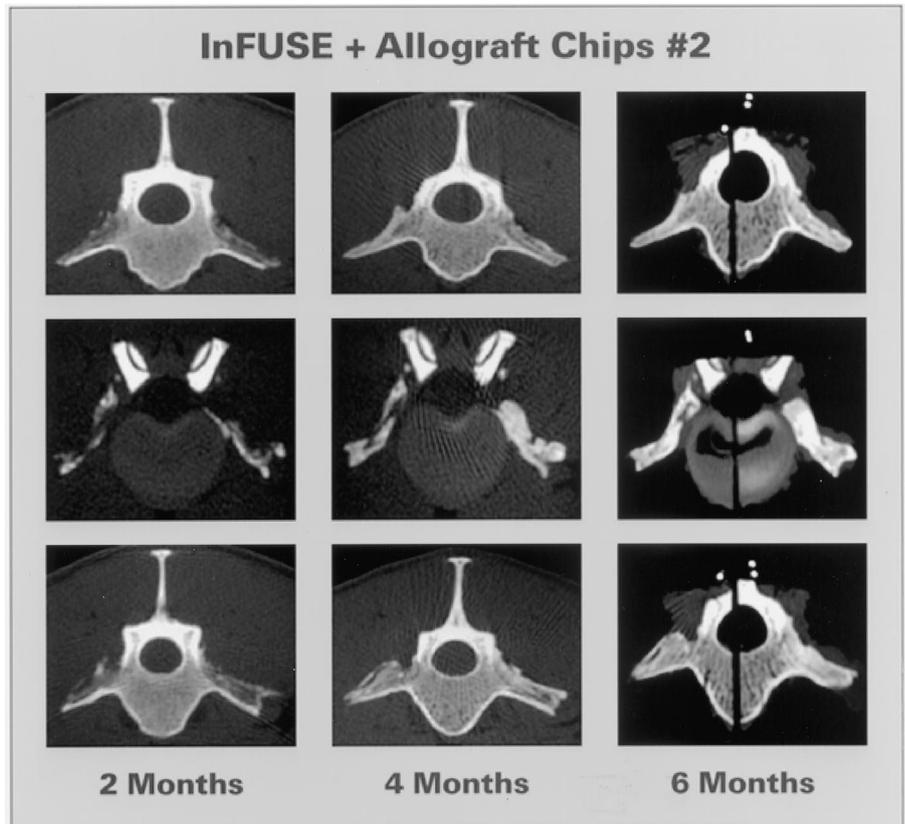
Figure 1. Representative axial computed tomographic scans from rhesus monkey lumbar spines 24 weeks after attempted posterolateral arthrodesis with autogenous iliac crest bone graft. The upper, middle, and lower panels in each column are representative images through the upper transverse process, intradiscal, and lower transverse process regions of the fusion, respectively. The three columns represent images at 2, 4, and 6 months moving from left to right. **A**, This series of images is from a monkey that had resorption of the bone graft, resulting in non-union. **B**, This series of images is from a monkey that had consolidation and successful healing of the bone graft, resulting in continuous bridging bone and solid fusion.

when delivered in a biphasic calcium phosphate (BCP) granule carrier; the ratio of hydroxyapatite (HA) to tricalcium phosphate (TCP) was 60:40.<sup>4</sup> The limitation of 60:40 BCP was its slow resorption time resulting from the high

HA content, making radiographic detection of new bone formation more difficult.

The purpose of this experiment was to evaluate the feasibility of enhancing the delivery of rhBMP-2 with the

Figure 2. Representative axial computed tomographic scans from rhesus monkey lumbar spines 24 weeks after attempted posterolateral arthrodesis with recombinant human bone morphogenetic protein-2 delivered in a collagen sponge enhanced with allograft bone chips. The upper, middle, and lower panels in each column are representative images through the upper transverse process, intradiscal, and lower transverse process regions of the fusion, respectively. The three columns represent images at 2, 4, and 6 months moving from left to right. A continuous bridge between vertebrae is seen, as well as a progressive increase in density (whiteness) within the fusion mass over time.



established collagen sponge carrier by adding faster-resorbing BCP granules (15:85) or allograft chips to provide compression resistance for the posterolateral lumbar environment.

## Materials and Methods

**Surgical Procedure.** Nine adult rhesus macaques (9–16 kg) underwent single-level posterolateral lumbar intertransverse process spinal arthrodesis. General anesthesia was induced with telazol (3–5 mg/kg intramuscularly/subcutaneously) and maintained with halothane (1–2%, inhalational). The monkeys were placed prone on chest rolls, shaved, prepared, and draped in a sterile manner.

The L4-L5 vertebral level was estimated by palpation of the iliac crests and comparison with a preoperative radiograph. A posterior midline incision was made to expose the lumbodorsal fascia after infiltration with 10 mL of bupivacaine local anesthetic as a supplement. Two longitudinal fascial incisions were made approximately 2 cm lateral of the midline. An intramuscular plane was developed between the multifidus and longissimus muscles to expose the transverse processes of L4 and L5 and the intertransverse membrane. The facet joints were left intact. Using a portable electric burr, the dorsal cortices of the transverse processes of L4 and L5 adjacent vertebrae were removed to provide a bleeding vascular bed for placement of the graft. The graft materials were prepared as described below and placed between the transverse processes in the paraspinous bed. The fascial incisions were closed with 3–0 absorbable sutures, and the skin was reapproximated with 3–0 absorbable sutures and skin staples.

Postoperative analgesia with bupimorphine (0.1 mg/kg) was given as needed. Animals were fed *ad libitum* and allowed to

move about their cages without restriction. No postoperative immobilization devices were used.

**Graft Materials.** The animals were divided into three groups, and in each group, one of the following materials was implanted between the transverse processes bilaterally.

1) *Autogenous bone graft* (n = 3). Corticocancellous bone was obtained through separate fascial incisions from each posterior iliac crest, cleaned of soft tissues, morselized, and placed over the decorticated transverse processes and intertransverse membrane (5.0 mL/side).

2) *Recombinant human bone morphogenetic protein-2 (3 mg) and 15:85 BCP granules (3 mL/side) in an absorbable collagen sponge* (Medtronic Sofamor-Danek, Memphis TN) (n = 3). A 2-mL bovine type I collagen sponge (1.85 × 3.5 × 0.35 cm) and 3-mL BCP granules soaked with 3 mg (2 mL of 1.5 mg/mL) rhBMP-2 solution per side (Medtronic Sofamor-Danek) were used. Biphasic calcium phosphate granules (Medtronic Sofamor-Danek) were made in a 15:85 HA-TCP ratio.

3) *Recombinant human bone morphogenetic protein-2 (3 mg) and allograft chips (5 mL/side) in an absorbable collagen sponge* (n = 3). A 2-mL collagen sponge (1.85 × 3.5 × 0.35 cm) and 5 mL allograft chips soaked with 3 mg (2 mL of 1.5 mg/mL) rhBMP-2 solution per side were used.

In the second and third groups, the ceramic granules or allograft chips were placed on the collagen sponge, wetted with the rhBMP-2 solution, and then rolled into a cylinder.

**Assessment of Animals.** All animals were killed at 24 weeks after surgery with intravenous pentobarbital, and the lumbar spines were removed. Spine fusion status was evaluated blindly

by manual palpation of the arthrodesed segment and by adjacent segments, posteroanterior radiographs, computed tomographic (CT) scans, and undecalcified histology.

**Manual Palpation.** At the time of harvest, the lumbar spines were manually palpated by a blinded observer at the level of the fused motion segment and at the levels of adjacent motion segments proximally and distally. Each motion segment was graded as solid or not solid (if any motion was present). Only levels graded as solid were considered fused.

**Radiographic Analysis.** Posteroanterior radiographs were made of all specimens. The tube-to-plate distance was 90 cm. The radiographs then were viewed, and the fusions were graded in a blinded fashion as solid or not solid based on the presence of a continuous trabecular pattern in the intertransverse fusion mass bilaterally.

**Computed Tomographic Scans.** All specimens underwent CT scans through and adjacent to the area of the arthrodesis at 8, 16, and 24 weeks after surgery. Scans were performed on a high-speed CT scanner (General Electric, Milwaukee, WI) with the following parameters: 100-cm field of view, 150 mA, 100 kV, 1-mm gap, and 1-mm slice thickness. Scans were evaluated for continuity of fusion mass and presence of bone in any unintended areas (either dorsally on the lamina or ventral to the intertransverse process membrane).

**Histologic Analysis.** The lumbosacral spine of each animal was fixed for 24 hours in 10% neutral-buffered formalin and then was placed in 70% ethanol. After fixation, the specimens were trimmed, dehydrated in 95% and 100% ethanol, and cleared in xylene. The undecalcified specimens were divided in the midsagittal plane, embedded in methylmethacrylate, and

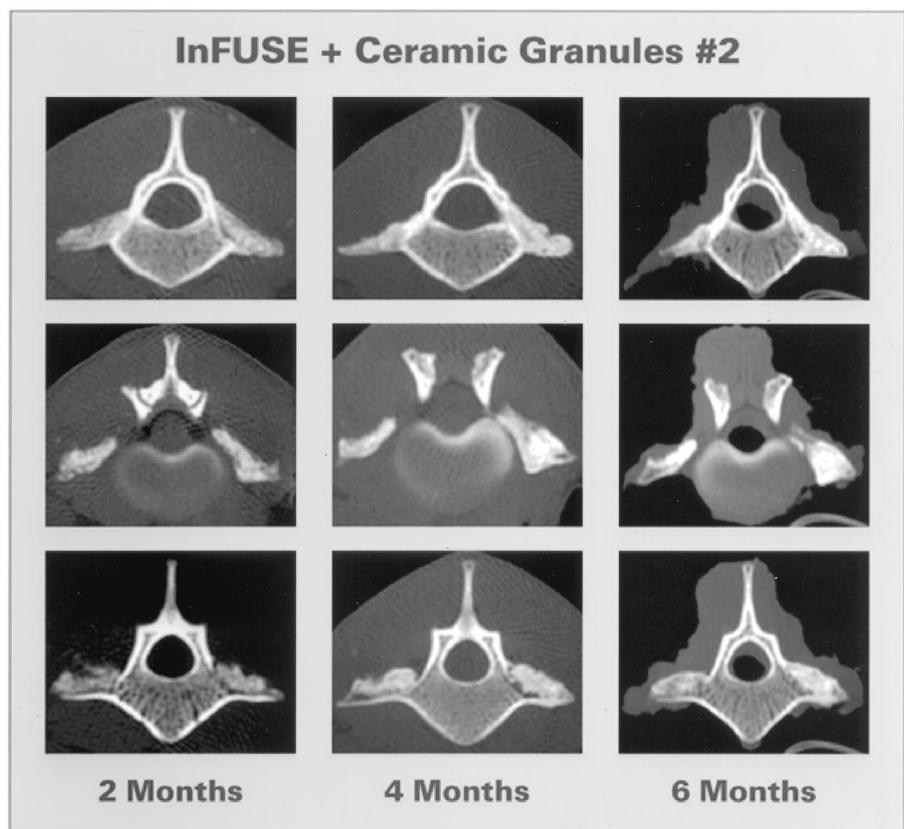
sectioned coronally or sagittally using an automated system (Exakt Technologies, Inc., Oklahoma City, OK) to an average thickness of 25  $\mu\text{m}$ . Contiguous sections were stained with 1% methylene blue and 0.3% basic fuchsin. Specimens were examined qualitatively for the extent of ingrowth trabecular bone and the presence of residual ceramic carrier. Spines were considered fused histologically when there was continuous bridging of new bone across the carrier connecting the two lumbar segments.

## ■ Results

All nine animals recovered from surgery uneventfully. One of the three animals that had autogenous bone as graft material achieved fusion, as judged by manual palpation. Plain radiographs revealed the continuous bone bridging the transverse processes bilaterally in the spine that was judged to be fused by palpation. Computed tomography scans showed all three autograft animals had progressive resorption of bone, but the one that achieved solid fusion had less resorption (Figures 1A and 1B).

Recombinant human bone morphogenetic protein-2 delivered in the collagen sponge augmented with 15:85 BCP granules or allograft chips induced spine fusion in six of six monkeys by manual palpation and CT scan analysis. Computed tomography scans showed that the amount of new bone in the fusion mass progressed in all rhBMP-2 cases between the 8-week and 16-week scans, evidenced by a progressive whitening of the fusion mass. There was less change between the 16-week scan and the final scan at 24 weeks (Figures 2 and 3). The new bone formation was con-

Figure 3. Representative axial computed tomographic scans from rhesus monkey lumbar spines 24 weeks after attempted posterolateral arthrodesis with recombinant human bone morphogenetic protein-2 delivered in a collagen sponge enhanced with 15:85 biphasic ceramic phosphate chips. The upper, middle, and lower panels in each column are representative images through the upper transverse process, intradiscal, and lower transverse process regions of the fusion, respectively. The three columns represent images at 2, 4, and 6 months moving from left to right. Although the fusion mass starts out already with some radiopacity because of the ceramic, a continuous bridge between vertebrae can be seen, as evidenced by the progressive increase in density (whiteness) within the fusion mass over time.



finned to the area in or adjacent to the carrier matrix; ectopic bone was not seen.

Histologic results paralleled those of the CT scans. One of the three autograft animals achieved a continuous bone bridge, but it was tenuous in the center based on histology (Figures 4A and 4D). All three of the BMP-2/allograft chip monkeys achieved solid fusions with normal bridging trabecular bone (Figures 4B and 4E). The allograft chips were completely resorbed. Osteoblast-lined trabeculae with sporadic osteoclasts and normal bone marrow elements were present. All three of the BMP-2/BCP monkeys also achieved continuous bridging bone, but a small amount of residual ceramic was still present (Figures 4C and 4F). Most of the ceramic had resorbed, but some was present and encased in normal bone, suggesting that the ceramic served as a scaffold and became incorporated with new bone before it could be resorbed. No bone was formed at a distance from the carrier.

## Discussion

The use of rhBMPs to achieve spine fusion has been successful in rabbits, dogs, and monkeys.<sup>5,6,11,19,20,22,27</sup> The delivery matrix can be as important as the choice of osteoinductive growth factor for bone induction. Previous studies have shown that a plain resorbable collagen sponge was a satisfactory carrier matrix for rhBMP-2 in rabbit and dog models of posterolateral intertransverse process arthrodesis. In nonhuman primate studies, the plain collagen sponge was susceptible to compression *in vivo* from the paraspinal musculature, which led to the requirement for higher doses of growth factor to achieve spinal fusion.<sup>17</sup> This compressibility problem required consideration of an alternative carrier. Although composite carriers can be engineered, the purpose of this study was to test simple additives to the plain collagen sponge that could be assembled at the time of surgery to enhance the compression resistance of the plain sponge.

In the present investigation, two additives to the collagen sponge were studied. The authors previously found that 60:40 HA-TCP proved to be a suitable carrier for rhBMP-2 in the posterolateral spine fusion model in rhesus monkeys, but it required 6–9 mg/side of rhBMP-2, and the 60% HA prevented radiographic detection of new bone formation. Therefore, for this study, the authors chose a BCP ratio of 15:85 HA-TCP to decrease the radiopacity and increase the resorption time of the ceramic. As an alternative to BCP granules, the authors tested the addition of allograft chips, which are readily available in most bone tissue banks.

Autogenous bone graft, which is the gold standard graft material, only led to solid fusion in one of three animals. This is comparable to earlier studies that showed poor healing with autograft alone in rhesus monkeys.<sup>5</sup> In contrast, all six rhBMP-2-treated animals achieved solid fusion with good quality fusion masses. Adding either allograft chips or 15:85 HA-TCP BCP

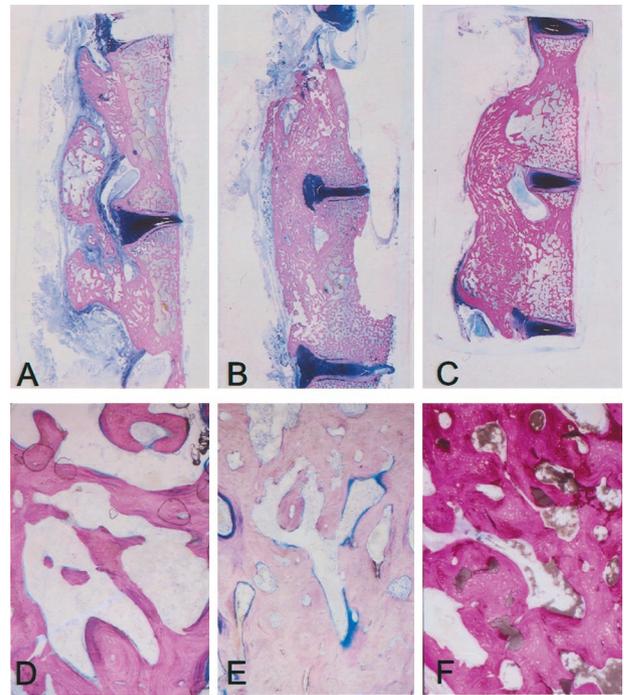


Figure 4. Representative whole-mount and medium-power (13.2× original magnification) photomicrographs from spine fusion masses in rhesus monkeys 24 weeks after posterolateral arthrodesis with autogenous iliac crest bone graft (A and D), recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered in a collagen sponge with allograft bone chips (B and E), or rhBMP-2 delivered in a collagen sponge with 15:85 hydroxyapatite-tricalcium phosphate ceramic granules (C and F). In all cases, normal bone can be seen with osteoblast-lined trabeculae and bone marrow elements. When rhBMP-2 was delivered with allograft bone chips, there was complete remodelling of the allograft bone (E). When rhBMP-2 was delivered with ceramic granules, a small of residual ceramic was seen encased in new bone formation (F).

granules to the collagen sponge afforded improved properties to the carrier matrix. In addition, a lower total dose of BMP-2 (3 mg/side) was successful, which compared favorably with the 6–9 mg/side that had previously been used when delivered in a 60:40 HA-TCP carrier. These findings suggest that addition of 15:85 HA-TCP or allograft chips to a plain collagen sponge may improve the economic feasibility of rhBMP-2 as a complete bone graft substitute. Another advantage of using 15:85 HA-TCP, rather than 60:40 HA-TCP, was the ability to detect new bone formation radiographically because of the higher ratio of TCP, which resorbed faster.

Only human trials can confirm efficacy of osteoinductive proteins in humans. However, the authors found that dose/carrier combinations involving BMPs in the rhesus monkey have been highly predictive of results in humans.<sup>4,5,7,17</sup> These data should be sufficient to justify human trials with the simple enhancements to the plain collagen sponge carrier matrix. Either of the two carrier enhancements tested here seem acceptable, although the BCP granules do not carry the theoretical risks associated with human tissue transplantation.

In summary, these data indicate that 3 mg/side of rhBMP-2 can be effective in posterolateral spine fusion in nonhuman primate when delivered on a resorbable collagen sponge enhanced by addition of either ceramic granules consisting of 15:85 HA-TCP or allograft bone chips. This study also highlights the importance of the carrier matrix for delivery of osteoinductive proteins, as well as the potential for the carrier to affect the required dose of protein.

### ■ Key Points

- In a nonhuman primate lumbar spine intertransverse model, arthrodesis with rhBMP-2 was more successful than with autograft as the graft material.
- A lower dose of rhBMP-2 was effective when the resorbable collagen sponge carrier was enhanced by addition of either 15:85 HA-TCP granules or allograft bone chips.

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