

Comparison of Posterolateral Lumbar Fusion Rates of Grafton Putty and OP-1 Putty in an Athymic Rat Model

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Study Design. Posterolateral lumbar spine fusions in athymic rats.

Objectives. To compare spine fusion rates of two different osteoinductive products.

Summary of Background Data. Many osteoinductive bone graft alternatives are available. Grafton (a demineralized bone matrix [DBM]) and Osteogenic Protein-1 (OP-1, an individual recombinant bone morphogenetic protein) are two such alternatives. The relative efficacy of products from these two classes has not been previously studied. The athymic rat spine fusion model has been validated and demonstrated useful to minimize inflammatory responses to xenogeneic or differentially expressed proteins such as those presented by DBMs of human etiology.

Methods. Single-level intertransverse process fusions were performed in 60 athymic nude rats with 2 cc/kg of Grafton or OP-1 Putty. Half of each study group was killed at 3 weeks and half at 6 weeks. Fusion masses were assessed by radiography, manual palpation, and histology.

Results. At 3 weeks, manual palpation revealed a 13% fusion rate with Grafton and a 100% fusion rate with OP-1 ($P = 0.0001$). At 6 weeks, manual palpation revealed a 39% fusion rate of with Grafton and a 100% fusion rate with OP-1 ($P = 0.0007$). Similar fusion rates were found by histology at 3 and 6 weeks. Of note, one or two adjacent levels were fused in all of the OP-1 animals and none of the Grafton animals.

Conclusions. Significant differences between the ability of Grafton and OP-1 to induce bone formation in an athymic rat posterolateral lumbar spine fusion model were found.

Key words: animal models, nude rats, bone morphogenetic proteins, demineralized bone matrix, spinal fusion, lumbar vertebrae. **Spine 2004;29:1612–1617**

Posterolateral lumbar fusion is a common surgical procedure that is used in the treatment of conditions including spondylolisthesis, spinal instability, and discogenic disease. Currently, autogenous iliac crest bone graft is the gold standard graft material against which all other graft materials must be compared.

Autograft, however, is associated with limitations of supply and donor site morbidities. Furthermore, pseudarthrosis rates with autograft vary from 5% to 35%.¹ Chronic donor site pain has been reported in 25% of patients.² Autograft availability may be limited by prior graft harvest or poor bone quality as well as the amount and shape of graft material feasible to recover. An additional operative site also increases blood loss, risk of infection, as well as operative time. As a result of these variables, the overall morbidity associated with iliac crest autograft in lumbar fusion approaches 30%.¹

Such limitations have prompted investigations into a variety of bone graft alternatives. The goals of such efforts are aimed at minimizing or eliminating the risks associated with autogenous bone graft while improving surgical outcomes. These products are osteoconductive if they provide a passive framework onto which new bone can be formed and/or osteoinductive if they provide molecular signals that actively increase bone formation.

Bone morphogenetic proteins (BMPs), members of the transforming growth factor- β superfamily, are the principle class of osteoinductive proteins. Initially described by Urist et al,^{3,4} these have now been characterized and produced in various forms for clinical use. When applied *in vivo*, BMPs induce undifferentiated mesenchymal stem cells to proceed down an osteogenic pathway.⁵ Demineralized bone matrix (DBM) and recombinant human bone morphogenetic protein (rhBMP) are both osteoinductive ability and may be osteoconductive (based on the carrier in which they are delivered).^{1,6–12}

DBMs are prepared by decalcifying allogeneic bone while leaving the extracellular matrix, which contains a relatively low concentration of constitutively expressed BMPs.¹ The amount and activity of these residual molecules depend, in part, on the methods of DBM preparation and preservation. A number of investigations have established the ability of some animal DBMs to induce fusion in animal models. Dog DBM has been shown to be an effective autograft extender in dog studies.^{7,9} Similar observations have been made with rabbit DBMs in rabbit models.^{1,10} Evaluation of human DBM products, however, is limited in animal models as these products

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Acknowledgment date: June 9, 2003. First revision date: September 14, 2003. Acceptance date: September 19, 2003.

Supported by a research grant from Stryker Biotech (Hopkinton, MA). The device(s)/drug(s) that is/are the subject of this manuscript is/are being evaluated as part of an ongoing FDA-approved investigational protocol (IDE) or corresponding national protocol for OP-1 long bone non union HDE approval.

Corporate/Industry funds were received to support this work. One or more of the author(s) has/have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this manuscript: e.g., honoraria, gifts, consultancies.

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have been shown to induce little or no bone formation in laboratory animals (xenogeneic models).¹³ It is thought that this failure of osteogenic activity results from immunologic responses of the recipient animal to nonconserved or differentially expressed proteins.

As a consequence of the above limitations, the athymic rat has been developed for assessing osteoinductive agents in a nonimmunogenic environment.^{14–17} Using this model, autograft fusion rates have been noted to be 30% (similar to immunologically intact rats), and no fusions were seen when no graft was placed.¹⁵ When a number of different commercially developed human DBMs were tested in this model, fusion rates varied greatly, from 0% to 75%.^{14,16}

In contrast, BMPs represent a class of highly conserved molecules that have been well-characterized, sequenced, and made available through recombinant techniques (rhBMPs [recombinant human bone morphogenetic proteins]). One such product is rhBMP-7, also known as osteogenic protein-1 (OP-1) (Stryker Biotech, Hopkinton, MA). Because individual BMPs are so highly conserved, there have not been problems testing these recombinant human products in animal models. Indeed, molecular analysis demonstrates greater than 90% homology between rabbit OP-1 and human OP-1.¹⁸ Consequently, animal studies with OP-1 have not demonstrated adverse inflammatory reactions,^{6,8} and OP-1 has been shown to induce high rates of fusion in rabbit and dog models.^{6,8}

We hypothesized that rhBMPs would induce significantly more fusions than DBMs in the athymic rat posterolateral lumbar fusion model because of the higher concentration of osteogenic molecules. To make this comparison, commonly available products from both classes of bone graft material were chosen: OP-1 Putty (Stryker Biotech) and Grafton Putty (Osteotech Inc., Shrewsbury, NJ).

■ Materials and Methods

Study Design. Single-level intertransverse process fusions were performed at L4–L5 (rats have six lumbar vertebrae) of 60 athymic nude rats (rnu/rnu). The animals were obtained from Harlan Sprague-Dawley (Indianapolis, IN) at 8 to 9 weeks of age. Thirty were grafted with Grafton Putty and 30 with OP-1 Putty. For each study group, deaths were performed at two time points: 3 and 6 weeks. This was based on work from Wang *et al* in which the maximum number of fusions was observed at 6 weeks when 2-, 4-, 6-, and 8-week time points were studied with athymic rat posterolateral lumbar fusions.¹⁷ After death, fusions were evaluated with radiographs, manual palpation, and histology. This protocol was approved by our institution's Animal Care and Use Committee.

Surgical Procedure. After acclimating at our facility for a minimum of 1 week, rats were induced with ketamine and xylazine (90 mg/kg and 4 mg/kg intraperitoneally, respectively) and anesthesia was maintained with isoflurane (0.5%–2%) and oxygen *via* a coaxial nose cone. Perioperative antibiotics were given (enrofloxacin 10 mg/kg subcutaneously). The rats were positioned and prepared in standard surgical fashion.

L4–L5 posterolateral fusions were then performed. The spine was approached through a single midline skin incision and two paramedian fascial incisions (Wiltse approach). The level was identified during surgery by referencing from the iliac crests. Once exposed, the transverse processes were decorticated; 2 cc/kg of either Grafton or OP-1 Putty was then placed in the fusion bed (3.5 mg OP-1, 1 g bone-derived Type I collagen, and 200 mg carboxymethylcellulose together produce 2.5 cc of OP-1 Putty). This equated to approximately 0.4 cc/animal or 0.2 cc/side. This dosing was drawn from work by Wang *et al* where 0.3 cc/side of Grafton Putty were used in a similar model.^{14,16,17} The dosing in the current study was decreased because of reports of renal toxicity secondary to the glycerol carrier of Grafton. In the quoted study, a 100% and 50% mortality rate was observed when 8 cc/kg and 4 cc/kg of Grafton Putty was used, respectively, as opposed to a 0% mortality rate when 2 cc/kg of Grafton Putty was used.¹⁹ For OP-1, the 2-cc/kg dose was higher than would be chosen to match the Grafton dose to allow direct comparison of outcome fusion rates.

Once the graft material was placed, the fascia was closed with 3.0 Vicryl sutures, and the skin was closed with staples. Incisions were dressed, and the animals recovered. For postoperative pain, one dose of Buprenex (0.03 mg/kg) was given once the rat was sternally recumbent. Enrofloxacin (50 mg/mL) and Tylenol (1 mg/mL) were added to the drinking water for the first 2 postoperative days. Dressings were removed after postoperative day 1. Half of the rats were killed at 3 weeks and half at 6 weeks.

Evaluation of Fusion. Posterior–anterior radiographs were taken at the time of death. Two reviewers evaluated the radiographs for fusion in a blinded fashion. Fusion was deemed present if bridging bone was noted in either intertransverse region. If there was disagreement in the reading of fusion by the two reviewers, a third reviewer evaluated the films to make the final determination of fusion.

The spines were then explanted, and manual palpation testing of the L4–L5 segment was performed. This has been found to correlate well with rigorous biomechanical testing in prior animal studies.⁸

Finally, the L4–L5 spinal segments were embedded in methylmethacrylate (MMA) for undecalcified histologic evaluation. Specimens were fixed in 70% ethanol, dehydrated in graded ethanols, and cleared in toluene under vacuum and pressure on a tissue processor (VIP 2000; Tissue Tek; Miles, Elkhart, IN). The undecalcified specimens were then infiltrated with increasing concentrations of MMA and embedded in MMA according to the method described by Baron *et al*.²⁰ Four-micron coronal sections were deplastified and then stained with toluidine blue (pH 3.7). Von Kossa staining was done on additional deplastified sections according to the technique described by Baron *et al*.²⁰ Histologic fusion was defined as bony trabeculae bridging from one transverse process to the next.

Evaluation of Renal Toxicity. Kidneys were harvested from both groups at both times of death, fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned. Sections of kidney tissue, 6 μ m in thickness, were mounted and stained with hematoxylin and eosin and evaluated histologically for evidence of tubular or parenchymal pathology.

Table 1. Manual Palpation Data

	No Graft*	Autograft*	Grafton	OP-1
3 wks	0/10 (0%)	2/10 (20%)	2/15 (13%)†	15/15 (100%)†
6 wks	0/10 (0%)	3/20 (30%)	6/15 (39%)†	15/15 (100%)†

*Baseline data previously defined for the athymic rat model.¹⁰

†Significant difference between Grafton and OP-1 groups at 3 and 6 weeks.

Table 2. Histology Data

	No Graft*	Autograft*	Grafton	OP-1
3 wks	0/10 (0%)	2/10 (20%)	2/15 (13%)†	15/15 (100%)†
6 wks	0/10 (0%)	3/20 (30%)	5/15 (33%)†	15/15 (100%)†

*Baseline data previously defined for the athymic rat model.¹⁰

†Significant difference between Grafton and OP-1 groups at 3 and 6 weeks.

Statistical Analysis. Fusion success rates as determined by manual palpation and histology between the different experimental groups were compared using the Fisher Exact Test. Results were considered significant at $P < 0.05$.

■ Results

The athymic rats used in this experiment weighed between 170 g and 200 g. The surgical procedures took approximately 20 to 30 minutes to perform. There were no complications, and all 60 animals survived until their assigned endpoints.

Manual palpation of the explanted spines revealed fusion rates as noted in Table 1. At 3 weeks, the Grafton Putty group had a fusion rate of 13% (2 of 15) and the OP-1 Putty group had a significantly higher fusion rate of 100% ($P = 0.0001$). At 6 weeks, the Grafton Putty group had a fusion rate of 39% (6 of 15) and the OP-1 Putty group had a significantly higher fusion rate of 100% ($P = 0.0007$).

Radiographic analysis revealed three fusions in the Grafton 3-week group and six fusions in the Grafton 6-week group. The OP-1 3-week group had 13 radiographic fusions while the OP-1 6-week group had 14 such fusions (Figure 1). Of the 60 films reviewed, there was disagreement in reading by the first two reviewers for five of the films. Three of these five were deemed to be not fused and two to be fused, by the third reviewer. Overall, with manual palpation as the gold standard for fusion, radiographs were 89% sensitive and 91% specific for fusion in this study.

Histologic analysis was very closely concordant with manual palpation results (Table 2), and there was no

significant difference between the two approaches ($P = 0.999$). At 3 weeks, histology revealed a 13% fusion rate for the Grafton group and a statistically different 100% fusion rate for the OP-1 group ($P = 0.0001$). At 6 weeks, histology demonstrated a 33% fusion rate for the Grafton group and a statistically different 100% fusion rate for the OP-1 group ($P = 0.0002$). The Grafton specimens that were interpreted as not fused revealed evidence of a cartilaginous matrix (parts of which had undergone ossification) without bridging between the transverse processes. The fused specimens demonstrated masses of new bone formation with clear bridging trabeculae from one transverse process to the next (Figures 2–5).

Of note, all OP-1 specimens demonstrated fusion at one or two adjacent levels by all three criteria above (manual palpation, radiography, histology). At 3 weeks, 12 of 15 specimens were fused at one adjacent level (10 were fused from L3 to L5 and two were fused from L4 to L6) and the remaining three were fused at both cephalad and caudad adjacent levels (L3–L6) by manual palpation. At 6 weeks, 4 of 15 specimens were fused at one adjacent level (L3–L5); the remaining animals were fused from L3 to L6 with the exception of one animal that fused from L4–S1 by manual palpation. Such an abundant response of new bone formation leading to adjacent level fusions was not seen in the rats treated with Grafton Putty.

All of the kidneys that were evaluated appeared histologically normal and were without evidence of glomerulonephritis.

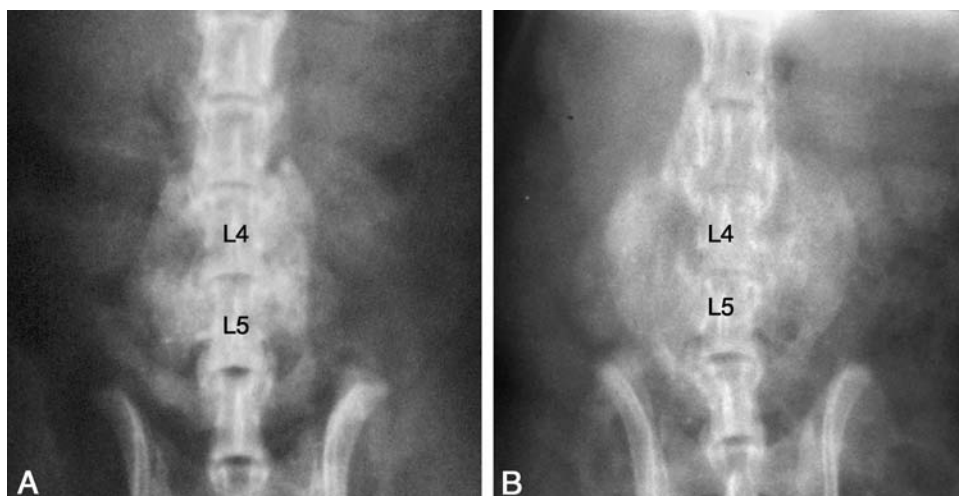


Figure 1. Radiographs of athymic rats grafted with Grafton (A) and OP-1 (B), which were judged to be fused 6 weeks after surgery.

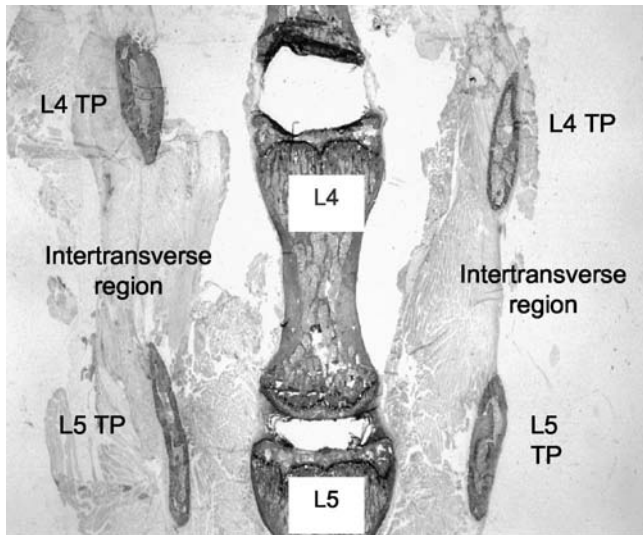


Figure 2. Coronal image of a toluidine blue stained histologic section of a control rat shown for orientation of anatomic landmarks. TP = transverse process.

■ Discussion

DBMs and rhBMPs represent two classes of bone graft materials currently being used in the clinical arena. Animal studies have demonstrated the ability of DBMs to act as bone graft extenders or enhancers^{1,7,10} and even as bone graft substitutes.^{14,16} In addition, animal studies have demonstrated the ability of rhBMPs to consistently induce fusion more rapidly and with greater success than autograft bone.^{8,21-25} A study directly contrasting the ability of a DBM and rhBMP to induce fusion has not previously been reported. This experiment compared posterolateral spine fusions induced by Grafton (a DBM) and OP-1 (a rhBMP) in an athymic rat model.

The athymic rat posterolateral fusion model has been characterized and validated. The absence of a thymus does not appear to have an effect on fusion rates when compared to normothymic rats and spontaneous fusions are not seen.¹⁵ In addition, the maximum frequency of spine fusions in this model occurs by the 6-week time point.¹⁷

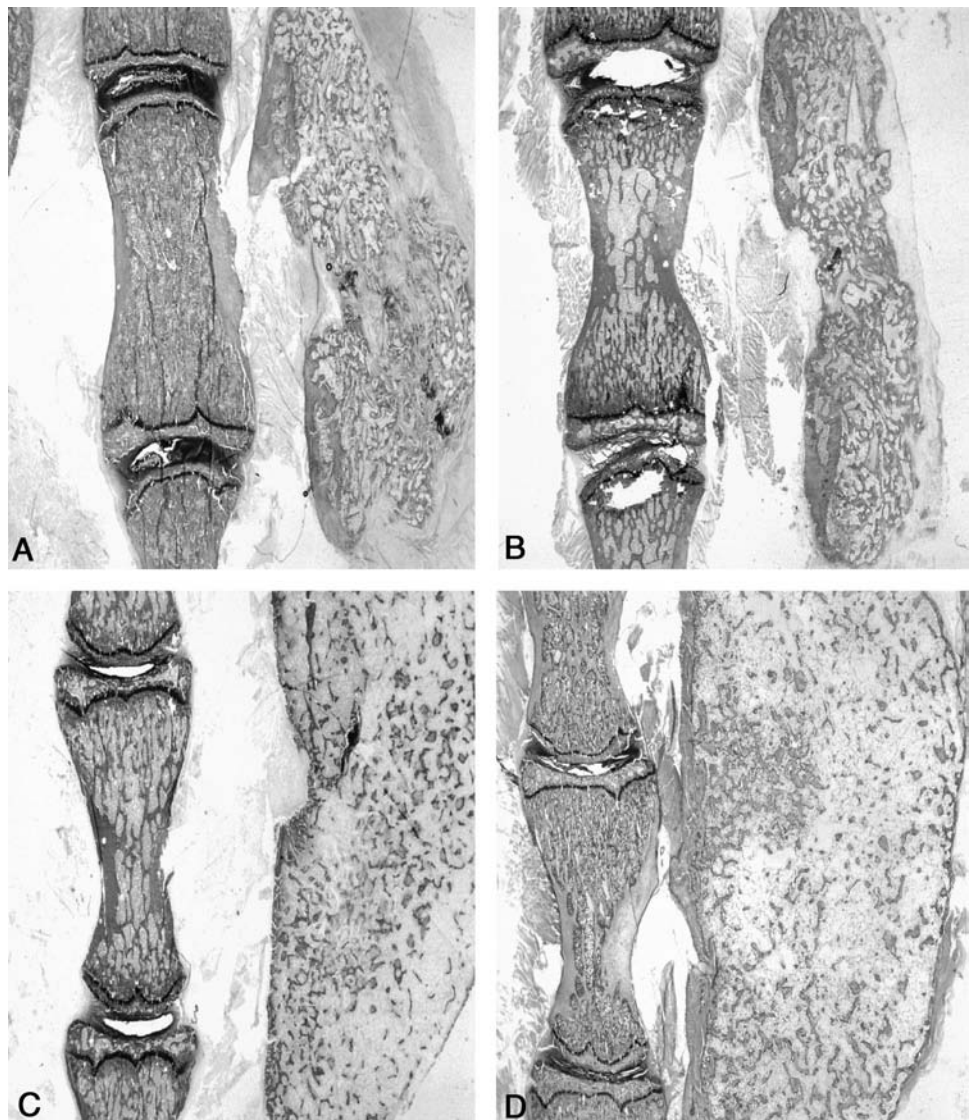


Figure 3. Toluidine blue staining of fused athymic rats grafted with Grafton at 3 weeks (A) and 6 weeks (B) and OP-1 at 3 weeks (C) and 6 weeks (D).

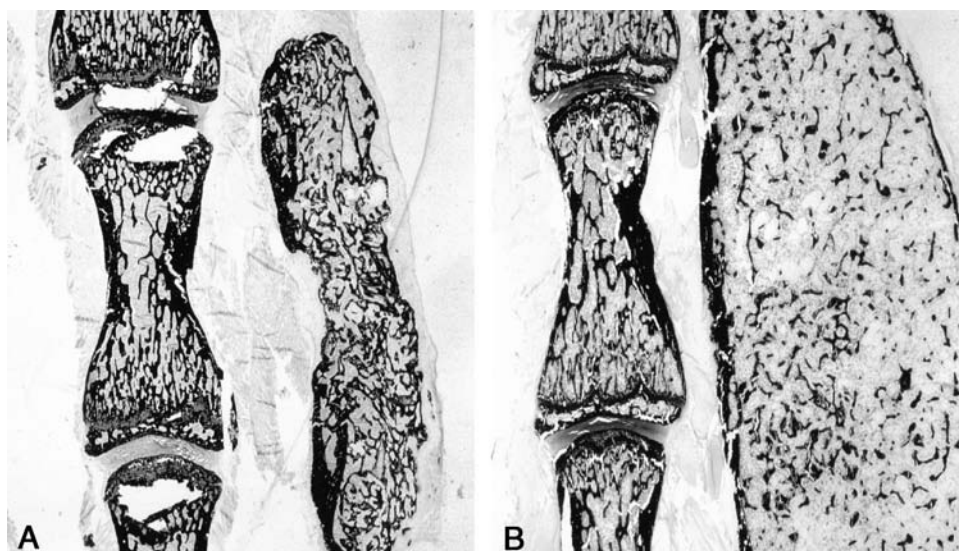


Figure 4. Von Kossa staining of 6-week fused Grafton (A) and OP-1 (B) specimens to highlight mineralization.

Multiple means of fusion assessment were used in this study. It is difficult to determine whether manual palpation or histology should be considered the gold standard for assessing fusion. Histology provides an accurate means of evaluating bone formation and quality, but it is possible to miss bridging bone that might cross out of the sectioned plane under study. Thus, it is highly sensitive for detecting fusion, but potentially less specific. Manual palpation has proven to be consistent with precise multidirectional biomechanical testing in prior studies.⁸ For these reasons, we chose to use manual palpation as the benchmark by which to determine fusion in this study. Our results demonstrate that OP-1 Putty induced fusion at a substantial and significantly higher rate than Grafton Putty in this athymic rat posterolateral lumbar spine fusion model.

We elected to use equal doses of Grafton and OP-1 Putty in this study, effectively eliminating a dosing bias.

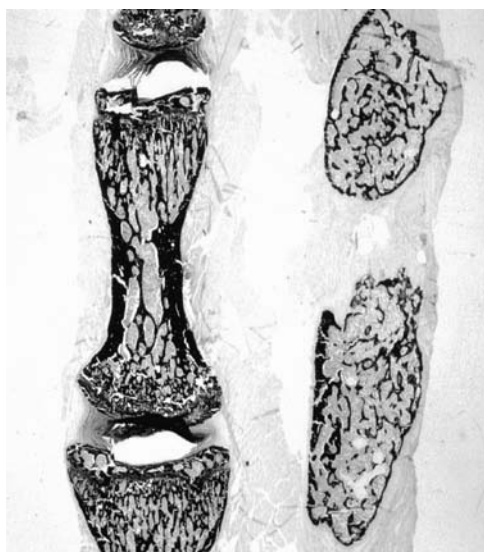


Figure 5. Von Kossa staining of 6-week Grafton specimen, which demonstrates failure of fusion.

A lower dose of Grafton was used than in prior studies in the rat to avoid glycerol toxicity from the Grafton carrier. Using this equal dosing, significantly more bone was formed with OP-1 than with autograft. It is unlikely that the extension of the fusion mass to nonintended levels resulted from carrier-related issues, as the carboxymethylcellulose/bovine collagen carrier is identical to those of other studies demonstrating fusion of only the intended levels.^{6,8} Simple inadvertent exposure of adjacent levels is also an unlikely explanation because adjacent level fusions would have been seen in the Grafton group as well. The high rate of fusion seen with Grafton and adjacent level fusions seen with OP-1 probably indicate that supraphysiologic doses of these agents were used. The absence of adjacent level fusions with OP-1 in the clinical setting, using more appropriate doses for humans, supports this conclusion.

The strengths of this study include that it is well controlled and used a validated animal model. Doses and endpoints were based on work from previous studies.^{14,16,17} Further, the manual palpation and histology results of this study were internally consistent.

There are limitations to this study. First, as with any animal fusion study, caution must be exercised when attempting to extrapolate these data to higher animal model, especially humans.²⁶ Second, these models do not reflect the range of pathology (age, osteoporosis, soft tissue injury) or deleterious systemic agents (steroids, malnutrition, smoking) that may be present in clinical situation.^{27,28} Finally, the differences in loading environments between quadrupedal animals and bipedal humans must be considered.²⁸

■ Conclusion

Grafton Putty (DBM) and OP-1 Putty (rhBMP) are directly compared. Results demonstrate impressive differences between the osteoinductive ability of Grafton and OP-1 in posterolateral lumbar spine fusions in athymic

rats. Further, the methods here offer a potential means of comparing other available osteoinductive agents.

■ Key Points

- Demineralized bone matrix (DBM) and recombinant human bone morphogenetic proteins (rhBMPs) are two classes of commercially developed bone graft materials with potential osteoinductive properties
- The efficacies of Grafton (a DBM) and osteogenic protein-1 (OP-1, a rhBMP) were compared in an athymic rat model.
- This model was chosen to minimize potential immunologic responses to human products which were being tested in an animal model.
- When equal quantities of the two products were compared, OP-1 induced significantly more bone formation and fusions than Grafton.

Acknowledgments

The authors thank Daniel Zelterman, PhD for his assistance with biostatistical analysis and Chris Coody for her assistance with histologic analysis as well as the Yale Core Center for Musculoskeletal Disorders.

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