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Controlling Bone Morphogenetic Protein Diffusion and Bone Morphogenetic Protein-Stimulated Bone Growth Using Fibrin Glue

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Study Design. An *in vitro* and *in vivo* study.

Objective. To evaluate the ability of fibrin glue to limit diffusion of recombinant human bone morphogenetic protein (rhBMP)-2 and its ability to protect spinal nerves from rhBMP-2 stimulated bone growth.

Summary of Background Data. Studies have shown bone morphogenetic protein (rhBMP-2) stimulated bone growth can encroach on the spinal canal and nerves, causing neural compression. More recently, rhBMP-2 use in the cervical spine has been associated with life-threatening swelling. Fibrin glue has been used as a biologic carrier but has not been evaluated for its ability to limit rhBMP-2.

Methods. In phase 1 of the study, rhBMP-2 soaked absorbable collagen sponges (ACS) were encapsulated in fibrin glue and immediately incubated in physiologic lactated ringers solution at 38°C. Samples of solution were tested for rhBMP-2 concentration. In phase 2 of the study, rats were surgically treated with laminectomy and placement of rhBMP-2/ACS *versus* laminectomy and placement of fibrin glue before placement of rhBMP-2/ACS. After 8 weeks, animals were euthanized and imaged using micro-computerized tomography.

Results. The diffusion study showed a significant limitation in rhBMP-2 diffusion when encapsulated in fibrin glue. The laminectomy study revealed blockage of bone formation by fibrin glue and protection of the spinal canal.

Conclusions. Fibrin glue can limit the diffusion of rhBMP-2, and, thus, it can be used to help protect the spinal canal and nerve roots from rhBMP-2 stimulated bone growth.

Key words: recombinant human bone morphogenetic protein-2, bone morphogenetic protein, fibrin glue, control, diffusion, bone growth stimulation. **Spine 2006;31:1201–1206**

Recombinant human bone morphogenetic protein-2 (rhBMP)-2 has been commercially available and recently approved for human spine use. It is rapidly becoming popular for augmenting fusion in the lumbar and cervical spine. Use of rhBMP-2 has been reported in anterior and posterior lumbar and cervical fusion surgery, as well as transforaminal lumbar interbody fusion (TLIF) and posterior lumbar interbody fusion (PLIF) type procedures. However, rhBMP-2 may stimulate bone growth in areas in which bone is not desired, especially as the material "leaks" into such spaces. The most detrimental effects of such heterotopic bone are growth into the spinal canal and neural foramina.[1–3]

In addition, cervical spine soft tissue swelling and airway compromise with rhBMP-2 use prompted a warning letter from Medtronic Sofamor Danek (Minneapolis, MN) of this significant risk. Although this phenomenon has not been thoroughly studied, it implies that the release of rhBMP-2 into the soft tissues stimulates a rapid, potentially life-threatening, inflammatory reaction. The swelling certainly warrants further study because it may be a carrier or bone morphogenetic protein (rhBMP-2) related, dose dependant, or even site specific. Thus, although our ability to stimulate bone growth rapidly increases with additional rhBMP-2s and other osteogenic agents, methods to control the possible adverse events associated with their use have not yet been presented or tested.

Fibrin glue has been used as a carrier of many osteoinductive materials, including rhBMP-2 and demineralized bone matrix,[4–8] as well as osteogenic cells.[9–11] It has also been used to improve the material handling of bone graft and bone graft substitutes.[12–14] However, conflicting reports show fibrin glue to augment[12] and inhibit[15–18] bone healing and bone formation. Although fibrin glue appears to limit rhBMP-2 diffusion,[5] its efficacy at modulating the clinical effect of rhBMP-2 or controlling its diffusion is not clearly known.

The purpose of this 2-phase study was to evaluate the use of fibrin glue to control the diffusion and bone forming effects of rhBMP-2. The first phase was an *in vitro* study of rhBMP-2 diffusion through fibrin glue. The second phase was to evaluate the ability of fibrin glue to protect the spinal canal and nerve roots from rhBMP-2 stimulated bone growth.

Although the results may have ramifications relative to soft tissue swelling, this study does not directly evaluate soft tissue swelling with or without the use of fibrin glue.

Materials and Methods

For all aspects of this study, commercially available Infuse® (Medtronic Sofamor Danek) brand rhBMP-2 was used at a

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concentration of 0.032 mg/mL, with the accompanying absorbable collagen sponge (ACS) cut to appropriate size (5 × 10 mm and 140 μL or 5 × 5 mm and 70 μL). This concentration of rhBMP-2 was used because it was the minimum concentration required for 100% successful stimulation of rat intertransverse process fusion (unpublished data, 2004). At this concentration, transverse process decortication and placement of rhBMP-2 containing sponges stimulate fusion 100% of the time, without the need for local bone or additional treatments, and, thus, this concentration of rhBMP-2 is known to stimulate bone formation in rats.

***In Vitro* Study.** Fibrin glue was tested for its ability to limit diffusion of rhBMP-2. Collagen sponges were soaked with rhBMP-2 in accordance with the package instructions (Infuse®). Of the 5 × 5 × 10 mm sponges in each group, 6 each were then submerged in 40 cc of normal saline under the following conditions: (1) control (no treatment), (2) placement of rhBMP-2 sponge on top of a fibrin glue layer, and (3) rhBMP-2 sponge sealed within a fibrin glue capsule. The vials were incubated at 37°C, and samples of the fluid were taken beginning immediately and at multiple times over 2 weeks. Fluid samples were analyzed for rhBMP-2 concentration using enzyme-linked immunosorbent assay (rhBMP-2 Quantikine ELISA kits; R&D Systems, Minneapolis, MN) and compared to the calculated maximum possible rhBMP-2 concentration if the collagen sponge were to release all of the rhBMP-2.

Animal Model. Female Lewis rats between 3 and 6 months of age were quarantined and observed for at least 7 days before surgical treatment. Rats were anesthetized with inhaled isoflurane before shaving and preparing. Surgical procedures were performed as described later, followed by postoperative monitoring. All wounds were closed with 4.0-nylon at both the fascial and skin layers. Immediately postoperatively, rats were given 1-cc lactated ringers solution, 0.2-cc Baytril (Bayer Healthcare LLC, Shawree Mission, KS) antibiotic, and 0.04-mL Buprenex (Pharmacal Inc., Richmond, VA) pain medication. Baytril was also given orally diluted (2 mL per 300-mL bottle) in the drinking water for 1 week. Rats were monitored for 8 weeks with periodic neurologic examinations before sacrifice.

Laminectomy Model. Laminectomies were performed at L1 and L5, taking care to minimize any trauma to the spinal canal and cord. No durotomies were noted during the course of the experiment. To assess bone growth encroachment into the spinal canal without attempting spinal fusion, each level after laminectomy was treated in alternating fashion, starting randomly in 1 of 2 ways: (1) placement of rhBMP-2 sponge directly over the dura (n = 9 rats); or (2) placement of fibrin glue on the dura, followed by at least 15 seconds curing time and then placement of the rhBMP-2 sponge (n = 9 rats) (Figure 1). In group 2, approximately 0.25 cc of fibrin glue was required to provide a smooth layer adequately over the dura. Separate fascial incisions were used for each laminectomy, and cross contamination was not allowed.

The rats were euthanized at 8 weeks, and spines were harvested for testing and imaging. Micro-computerized tomography (CT) was performed, and images were analyzed for bone formation, spinal canal diameter, and spinal canal area. The measurements were compared to the untreated L3 level spinal canal.

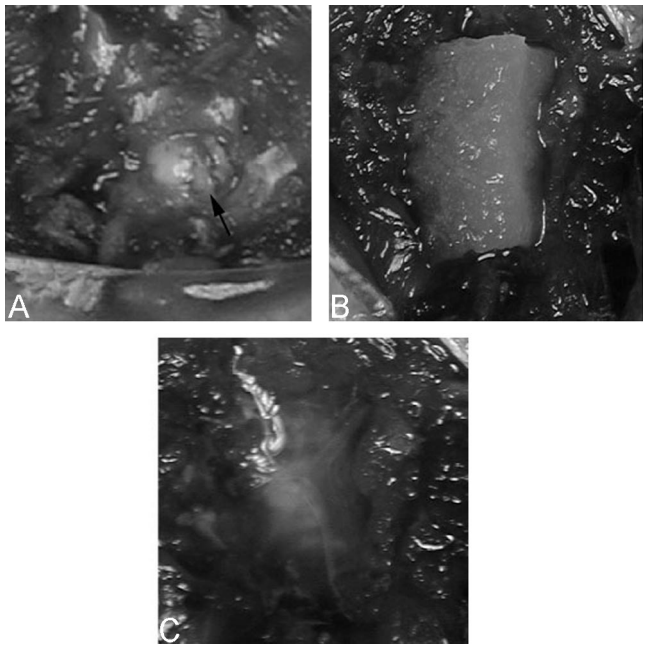

Figure 1. Rat surgery laminectomy. **A**, Rat spine exposed with completed laminectomy and dura exposed (arrow). **B**, Laminectomy with rhBMP-2 sponge placed directly over dura. **C**, Laminectomy with fibrin glue placed and before placement of rhBMP-2 sponge.

■ Results

In Vitro *Study*

The rhBMP-2 concentration in the solution as a function of time is shown in Figure 2. The control and fibrin glue with rhBMP-2 on top curves show a rapid increase to relatively steady high levels of rhBMP-2 in the solution. Statistically, the control (rhBMP-2 sponge only) and rhBMP-2 sponge on top of fibrin glue results were not significantly different ($P > 0.10$) at all times; however, both were significantly higher ($P < 0.05$) than the rhBMP-2 encapsulated in fibrin glue curves at all times

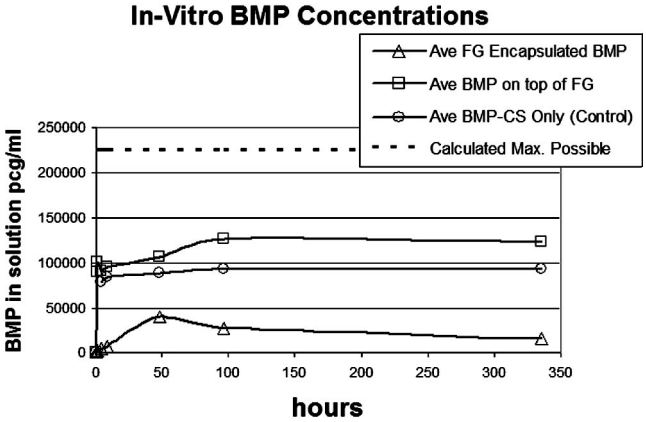

Figure 2. Diffusion of rhBMP-2 through fibrin glue (FG). Graphs show averages (Ave) of control with just rhBMP-2-collagen sponge (rhBMP-2-CS), rhBMP-2 sponge on top of layer of fibrin glue, and rhBMP-2 sealed in fibrin glue capsules. Note slow increase and significantly lower peak concentration of rhBMP-2 in solution when encapsulated with fibrin glue. Calculated maximum (Max) possible concentration is shown at the top. Pcg, picograms.

except at t = 0 and 1 minute. In addition, the fibrin glue does not appear to bind the rhBMP-2 because the curve showing the rhBMP-2 on top of the fibrin glue has a steady high level similar to the rhBMP-2 sponge only curve. The curves of the rhBMP-2 sealed in the fibrin glue capsules show a slower, steady increase over 4 days and a much lower peak concentration than the control groups. None of the controls or experimental groups showed levels close to the calculated maximum possible rhBMP-2 concentration, which is likely because a significant amount of rhBMP-2 remained bound to the collagen sponge. Conversely, it is noteworthy that a significant portion of the rhBMP-2 did rapidly diffuse into the solution when the sponge was not encapsulated.

Animal Model

No abnormal rat behavior was noted, and no rats appeared to have an abnormal postoperative course. No rats in this study died. None of the rats showed any neurologic deficits before or after the surgical procedure, or at sacrifice.

Laminectomy Model

Manual testing of rats treated with laminectomies revealed that although intertransverse fusion was not attempted, 2 of the 9 spines treated with rhBMP-2 without fibrin glue fused, and none of the 9 laminectomy spines treated with fibrin glue before placement of rhBMP-2 fused. Radiographs revealed at least partial bone formation at the spinal canal edges in all cases. Micro-CT imaging of rats treated with rhBMP-2 alone revealed decreased spinal canal diameters in anteroposterior dimension relative to adjacent untreated levels ($P < 0.05$), while the cross-sectional area trended lower but was not statistically significant (Figure 3, Table 1). Rats treated with rhBMP-2 and fibrin glue showed either no bone formation over the mid portion of the laminectomy (Figure 4) or diameters higher than adjacent untreated levels ($P < 0.05$), and cross sectional area measurements higher than adjacent levels ($P < 0.05$; Figure 5, Table 1).

■ Discussion

Although many animal and clinical studies have proven the safety of rhBMP-2 use near the spinal canal, significant concerns remain. Rabbit and mouse models have shown that rhBMP-2-induced calcification of the ligamentum flavum can lead to significant spinal stenosis and myelopathic changes.[19–21] This raises concerns that rhBMP-2 leakage, especially in the cervical canal, may increase ossification of the posterior longitudinal ligament. In a dog study, Meyer *et al*[22] showed that rhBMP-2 can stimulate bone formation in a laminectomy defect when placed directly over the dura. They also reported concavity of the posterior canal in some instances, although this resolved to the normal convexity by 3 months. In addition, although they did not report any long-term spinal canal stenosis, there was a continued decrease in foraminal size in the rhBMP-2 treated animals that did not resolve over time.[22]

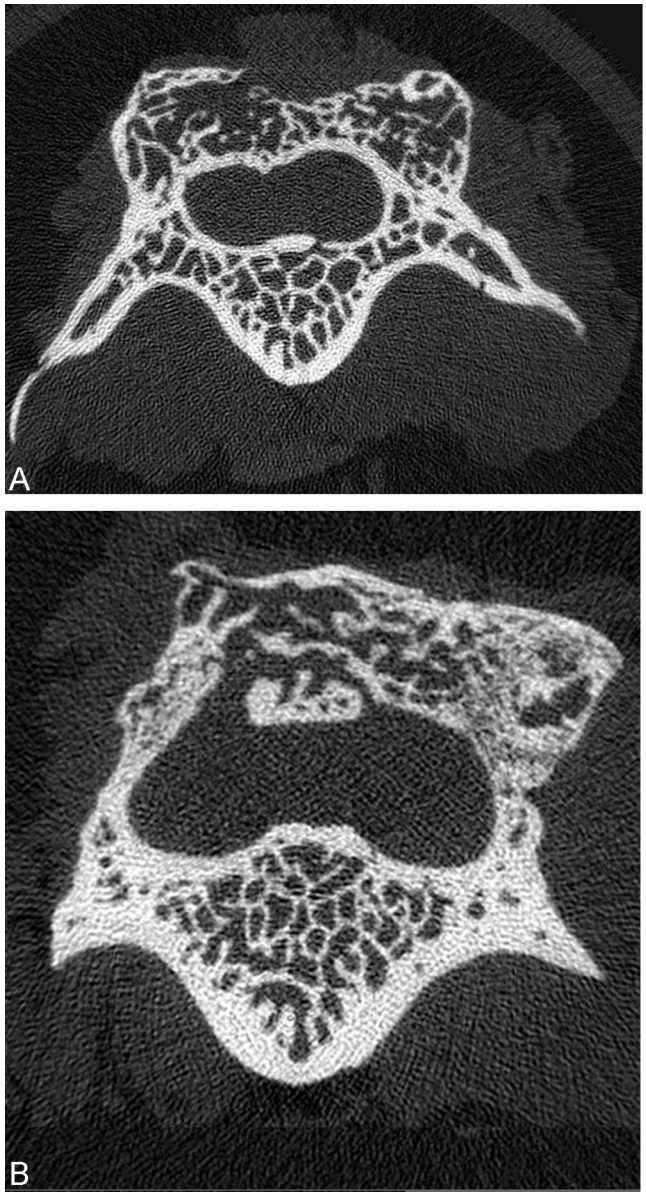

Figure 3. Micro-CT images of laminectomy with rhBMP-2 only. Examples with rhBMP-2 sponge placed and no fibrin glue. Note significant narrowing of spinal canal and copious bone formation in these two typical specimens A and B.

Finally, in humans, Haid *et al*,[3] aborted a study using cages in a PLIF approach because of bone formation along the track of cage insertion, including within the spinal canal. In that study, 24 of 34 patients had bone

Table 1. Laminectomy Study Anterior-Posterior and Area Measurements

	Fibrin Glue + rhBMP-2		rhBMP-2	
	Anterior-Posterior Distance (cm)	Area (cm^2)	Anterior-Posterior Distance (cm)	Area (cm^2)
Treated	2.33*	4.44*	1.27*	3.14
Adjacent	1.48*	3.48*	1.49*	3.24

*Denotes statistically significant difference of treated relative to adjacent levels ($P > 0.05$).

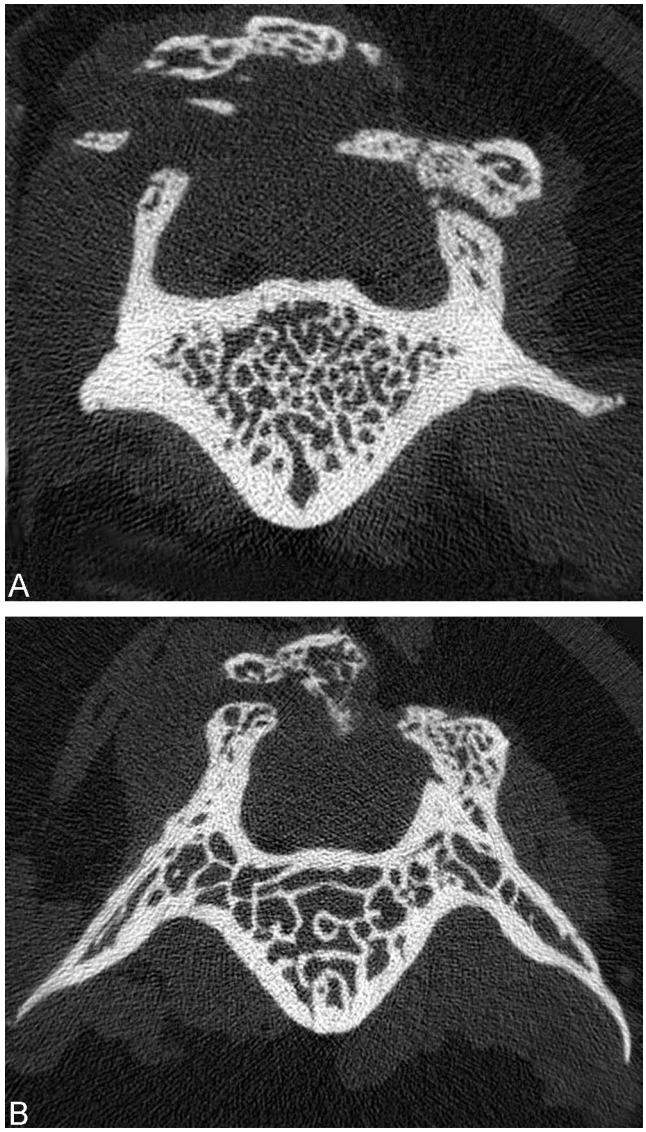

Figure 4. Micro-CT images of laminectomy with fibrin glue placed before rhBMP-2. Laminectomy examples with fibrin glue placed before rhBMP-2 sponge. Note larger canal area and wide open canal in these two typical specimens A and B.

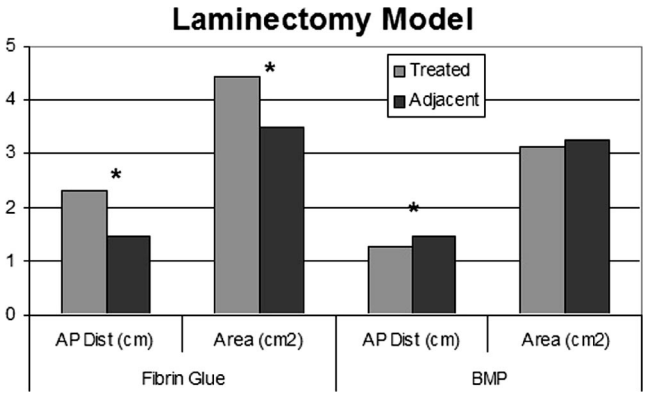

Figure 5. Anterior-posterior distance (AP Dist) and spinal canal area measurements for laminectomy with and without fibrin glue placed before rhBMP-2 sponge. Asterisk (*) denotes statistical significance ($P < 0.05$) between treated level and adjacent level.

formation in the spinal canal, while only 4 of 33 autograft controls had similar bone formation. Thus, rhBMP-2 does have the potential to cause significant nerve and canal compromise. More recent reports of cervical soft tissue swelling and postoperative airway compromise prompted a warning letter from Medtronic Sofamor Danek that rhBMP-2 should be used with extreme caution in the cervical spine, which implies that rhBMP-2 can stimulate a rapid soft tissue inflammatory response. Thus, we not only have to protect the spinal canal and foramina from abnormal bone growth but also the soft tissues from excessive swelling.

Fibrin glue has a long history of use in surgical applications involving bone healing. It was first used as a clotting substance in 1909, and the modern version was developed in Vienna in 1972. It has 2 components, fibrinogen and thrombin; when mixed, the thrombin activates the fibrinogen to form a fibrin clot. This clot material was thought to stimulate bone formation in some human and rat studies,[12,23] although other studies refute that it has a positive effect, and it may even have a mild inhibitory effect depending on how it is used.[15–18,24,25] At the same time, it did not appear to have a negative effect on bone healing or bone formation with other studies,[24,26] and its gelatinous consistency has been used to improve bone and bone substitute handling.[7,14,26] It has even been used in kyphoplasty type applications with new filler materials.[13] In the end, it seems uncertain whether it stimulates, inhibits, or does not affect bone formation directly.

Newer studies have successfully used fibrin glue as a carrier for osteogenic cells,[10,27] and even rhBMP-2.[4,28,29] In the study by Nakamura,[6] fibrin glue, rhBMP-2, and autograft bone were implanted at pars defects in rats, resulting in exuberant bone formation. In a mouse study, Hattori[5] postulated that fibrin glue may be useful in controlling the diffusion and delivery of rhBMP-2 when it controlled the area of osteochondrogenesis. Thus, there is evidence that fibrin glue can control the diffusion of rhBMP-2 without significantly altering its effectiveness.

In the *in vitro* aspect of this study, rhBMP-2 was gradually released from the fibrin glue capsule. Although it does not completely block the rhBMP-2, it does appear to impede significantly the diffusion of rhBMP-2. The fact that the curves of the control samples and the samples with the rhBMP-2 placed on top are very similar implies that the rhBMP-2 was also not bound or significantly altered by the fibrin glue. This is important because a sealant used to protect the neural elements should also not act as a rhBMP-2 sink.

Interestingly, in the nonencapsulated samples, the collagen sponge released nearly half the rhBMP-2 almost immediately, which implies that this rhBMP-2 was not bound but simply held in the sponge's absorbed fluid, allowing nearly immediate diffusion into solution. This effect appears to validate concerns of fluid and, thus, rhBMP-2 leak-

age from the sponge into the spinal canal and foramina. Indeed, in a clinical study, when the fluid that dripped from collagen sponges used for anterior cervical fusions was tested, significant amounts of rhBMP-2 were found.[30]

The laminectomy model shows the usefulness of fibrin glue. Similar to the study of dogs by Meyer *et al*,[22] the laminectomy rats with rhBMP-2 placed directly over the dura did not show any signs of neurologic compromise. However, the spinal canal diameters in these animals were significantly narrowed compared to untreated levels, with a trend toward decreased canal areas. The fibrin glue placed over the dura appears to have acted as a buffer, keeping the bone formation away from the canal. Whether the mechanism of control of bone formation was from blocking the rhBMP-2 from reaching the osteogenic cells or from blocking the osteogenic cells from reaching the rhBMP-2 is uncertain. Regardless, the fibrin glue significantly disrupted the connection.

It could be argued that the fibrin glue itself could cause compression of the spinal canal. Although this possibility was not examined in this study, it is very unlikely because fibrin glue is metabolized just as a coagulated hematoma. Thus, it would not cause any more initial compression than low-pressure bleeding, and it completely disappears over the course of the first few weeks after a procedure.

Whether or not fibrin glue would be useful in the cervical spine to decrease the risk of soft tissue swelling is difficult to ascertain. If the inflammation is dose dependant, then fibrin glue may decrease the diffusion enough to make rhBMP-2 use safer. However, if it is related to the carrier or simple presence of the rhBMP-2, then it may have no effect. Further studies are certainly necessary.

It is also noteworthy that the amount of bone formation appeared significantly reduced in the fibrin glue specimens on subjective evaluation. This reduction in bone formation should caution the user of fibrin glue to keep it separated from decorticated bone surfaces, where bone formation actually is desired. For example, the fibrin glue could be used to cover the dura in a posterior-lateral fusion, while keeping it away from the decorticated facet joints and transverse processes. Similarly, it may be used to "seal" the anular window in a TLIF or PLIF procedure, without significantly entering the interbody space. This latter application was not possible in the animal model and should be approached cautiously by the clinician, especially in keeping the fibrin glue separated from the fusion surfaces.

This study does have several limitations. The rat model is certainly not the same as a human model, and bone formation is not as easy to stimulate in human beings as in rats. In addition, the rhBMP-2 concentration used was the minimum required for 100% fusion in a rat fusion model. The commercially available Infuse® product rhBMP-2 concentration is much higher and may diffuse through the fibrin glue in high enough concentrations to stimulate bone formation. Studies with higher concentrations in rat models are underway, but, because bone stimulation thresholds are higher in human beings, the answers will not really be elucidated until clinical human studies are performed. In addition, it was not possible to create a TLIF or PLIF model in rats, whereas, perhaps in a larger animal, this could be performed. Thus, the reader should be cautioned against assuming fibrin glue is an ideal solution to the potentially adverse events associated with rhBMP-2 use.

Overall, fibrin glue does not appear to bind or adversely affect the function of rhBMP-2 when placed adjacent to rhBMP-2 soaked collagen sponges. Fibrin glue does limit but does not completely stop the diffusion of rhBMP-2. Fibrin glue can limit the formation of rhBMP-2 stimulated bone growth and, thus, protect the spinal canal from undesired bone formation.

■ Key Points

- Fibrin glue can limit rhBMP-2 diffusion.
- Fibrin glue can protect the spinal canal from encroachment of rhBMP-2 stimulated bone growth.
- Fibrin glue can control rhBMP-2 stimulated bone growth.
- A large proportion of rhBMP-2 rapidly diffuses out of the collagen sponge when it is not controlled with a substance such as fibrin glue.

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