Efficacy of interspinous process lumbar fusion with recombinant human bone morphogenetic protein-2 delivered with a synthetic polymer and β -tricalcium phosphate in a rabbit model

Tomiya Matsumoto · Hiromitsu Toyoda ·
Sho Dohzono · Hiroyuki Yasuda · Shigeyuki Wakitani ·
Hiroaki Nakamura · Kunio Takaoka

Received: 11 March 2011 / Revised: 2 December 2011 / Accepted: 18 December 2011 / Published online: 28 December 2011
© Springer-Verlag 2011

Abstract

Introduction As a powerful bone-inducing cytokine, rhBMP-2 has been used as a bone graft substitute in combination with animal-derived collagen to achieve interbody or posterolateral spinal fusion. Successful interspinous process fusion using rhBMP-2 in combination with synthetic carrier materials would offer a safe, minimally invasive spinal fusion option for the treatment of spinal disorders. The aims of the present study were to achieve interspinous process fusion by implanting rhBMP-2-retaining degradable material instead of bone grafting and to evaluate efficacy for vertebral stabilization.

Materials and methods A polymer gel (200 mg), β -tricalcium phosphate powder (400 mg), and rhBMP-2 (0, 30, 60 or 120 μ g) were mixed to generate a plastic implant, which was then placed during surgery to bridge the L5–6 interspinous processes of 58 rabbits. Control animals received implants either without rhBMP-2 or with autogenous bone chips from the iliac crest. L5–6 vertebrae were recovered 8 weeks postoperatively. Interspinous process fusion was evaluated by radiography, biomechanical bending test, intradiscal pressure (IDP) measurement, and histology.

Results In bending tests, strength of fusion was significantly greater in BMP60 and BMP120 groups than in sham, BMP0, BMP30 or autogenous bone groups. IDP at L5–6 was significantly reduced in BMP60 and BMP120 groups compared to sham, BMP0, BMP30, and autograft

groups. Histologically, coronal sections of the fusion mass showed a bone mass bridging both spinous processes.

Conclusion Solid interspinous process fusion was achieved in rabbit models by 8 weeks after implanting the biodegradable bone-inducing material. These results suggest a potential new less-invasive option without bone grafting for the treatment of lumbar disorders.

Keywords Animal model · Interspinous process spine fusion · Minimally invasive surgery · Recombinant human bone morphogenetic protein-2

Introduction

Anterior or posterolateral spinal fusion with autogenous bone grafting has been a routine method for the treatment of spinal disorders associated with spinal instability caused by degenerative changes or trauma to the spine. Although several studies have shown satisfactory outcomes from spinal fusion for such spinal disorders, risks of complications associated with the extensive surgery remain, particularly in old patients [18, 28, 30]. In this context, development of less-invasive spinal fusion options is desirable to treat spinal disorders.

In terms of less-invasive spine surgery, the close proximity of the spinous processes to the skin allows for the implantation of interspinous process devices with minimal operative invasiveness. A large number of interspinous process devices have recently entered the market, some of which are promoted to facilitate fusion, while others are designed to preserve mobility. Interspinous process devices such as X-stop, ExtenSure, and Wallis implants have been used in operative methods for lumbar spinal stenosis in recent years. The principle underlying interspinous process

T. Matsumoto · H. Toyoda (✉) · S. Dohzono · H. Yasuda ·
S. Wakitani · H. Nakamura · K. Takaoka
Department of Orthopaedic Surgery, Osaka City University
Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku,
Osaka 545-8585, Japan
e-mail: h-toyoda@msic.med.osaka-cu.ac.jp

devices is to unload the facet joints, restore foraminal height, and provide stability in order to improve the clinical outcomes of surgery [6]. Interspinous fixation has also been attempted to avoid the risks associated with transpedicle or sublaminar instrumentation. The most rudimentary form of interspinous fixation involves wiring the spinous processes, and the Daab plate and Wilson plate have since been developed [7, 9, 13]. Spinal instrumentation using the spinous process as an anchor offers limited flexibility [14], but recent studies of lumbar fusion surgery using the Tadpole system and CD Horizon Spire spinous process plate[®] have shown relatively favorable clinical outcomes [19, 41].

The concept of interspinous process fusion is a classical one and the first animal model used to study posterior interspinous spinal fusion dates back to Albee's experiments in 1913 with autogenous bone grafting in dogs [2]. Oikarinen [27] and Lindholm [23] later reported interspinous process fusion without bone autograft in rabbits. Oikarinen [27] achieved interspinous process fusion with allogeneic decalcified bone matrix. Lindholm [23] reported the combined use of allogeneic decalcified bone matrix and autogenous bone marrow with a fusion rate of 86% in 20 weeks.

However, posterior spinal fusion has rarely been indicated in clinical practice due to the high incidence of pseudoarthrosis and the long time required to obtain complete fusion even with use of adequate amount of autograft [11, 37]. To address these issues, we have introduced recombinant bone morphogenetic protein (BMP)-2 and have developed a delivery system [20, 21, 24, 34] to enhance new bone formation temporospatially and avoid surgery to procure autograft material. The properties of the delivery system as a carrier material can be listed as follows: (1) no risk of disease transfer; (2) controlled biodegradability that can effectively regulate the release of recombinant human BMPs (rhBMPs); and (3) plasticity and ease of handling [21]. The present experimental study attempted a simple and less-invasive spinal fusion of posterior interspinous processes using rhBMP-2 in combination with synthetic carrier materials without bone grafting. Solidity of the fused spinal segment was then examined using radiological, histological, and biomechanical methods. We consider that achievement of solid interspinous process fusion could reinforce biomechanical weakness in patients with vertebral fracture, disc degeneration, degenerative spondylolisthesis, spondylolytic spondylolisthesis or inter-body fusion.

Materials and methods

rhBMP-2

The *Escherichia coli*-derived rhBMP-2 (E-BMP-2) used in this study was produced and kindly donated by Osteopharma

(Osaka, Japan) [43]. E-BMP-2 with a dimeric molecular structure was produced in human BMP-2 gene-transfected *E. coli* with a monomeric structure and stored in inclusion bodies, which were collected. The molecular structure was unfolded using protein-denaturing agents, and then refolded to form dimeric E-BMP-2 on removal of the denaturing agents. Dimeric E-BMP-2 was purified using several steps of chromatography. Details of the procedures for dimerization of cytokines have already been reported [12, 32, 42, 43].

Poly-D,L-lactic acid/polyethylene glycol block copolymer (PLA-PEG)

PLA-PEG (molecular weight 9,500) was provided by Taki Chemical (Kakogawa, Japan). The physicochemical characters and efficacy of the hydrogel polymer for local delivery of BMP-2 to elicit ectopic bone formation has been reported elsewhere [21].

Porous β -tricalcium phosphate (β -TCP) powder

The β -TCP powder (particle diameter, 0.5–1.5 mm; porosity, 75%) was manufactured and provided by Olympus Optics (Tokyo, Japan).

Preparation of bone graft substitute implants

To prepare an implant (Fig. 1a) to bridge L5 and L6 interspinous processes in one animal, freeze-dried E-BMP-2 powder was dissolved in distilled water to reconstitute before use under sterile conditions. 400 mg of β -TCP powder, 200 mg of PLA-PEG, and one of three dosages of rhBMP (30, 60, or 120 μ g) were mixed by stirring with a metal rod at 50°C for several minutes to liquefy the polymer. As control implants, 400 mg of β -TCP granules and 200 mg of PLA-PEG without rhBMP-2 were prepared in the same manner. The prepared implants were stored at –30°C until implantation. We tested the heat tolerance of E-BMP-2 in a previous study and confirmed that this sterilization process does not affect the activity of rhBMP-2 [43].

Surgery and experimental protocols

Fifty-eight Japanese white rabbits (18 weeks old; weight 2.8–3.2 kg) were divided randomly into six groups (Table 1) depending on the dose of BMP-2 in implants (BMP30, BMP60, BMP120 and BMP0), along with an autografted group and a sham-operated group. Another five rabbits died under anesthesia or during follow-up. However, no animal deaths related to materials were encountered.

Fig. 1 **a** BMP-2-retaining paste-like implant. The implant for an animal was prepared by mixing a polymer gel (200 mg), β -TCP powder (400 mg) and rhBMP-2 (0, 30, 60 or 120 μ g). **b** Implantation to bridge L5–L6 spinous processes. *Right cranial*

Table 1 Composition of implant in each group

Group	rhBMP-2 (μ g)	β -TCP (mg)	PLA-PEG (mg)	Number
Sham	0	0	0	7
BMP 0	0	400	200	7
BMP 30	30	400	200	11
BMP 60	60	400	200	11
BMP 120	120	400	200	11
Autogenous bone	Autogenous iliac bone graft (2.4–2.9 g)			11

Each animal underwent histological evaluation

Before surgery, animals were anesthetized with an intramuscular injection of ketamine (30 mg/kg) and xylazine (10 mg/kg). Cefazolin (100 mg) was administered subcutaneously as a prophylactic antibiotic. Each rabbit underwent surgery for a single level interspinous process fusion at L5–L6 level. After making a dorsal midline skin incision and a median fascial incision, the paraspinal muscles were retracted to expose the spinous processes of L5 and L6 and the interspinous ligament. An electric burr (Stryker, Kalamazoo, MI) was used to decorticate the cortex on both sides of the L5 and L6 spinous processes. The implant was a paste-like material with easy handling and molding characteristics. The volume of one implant was 0.45 ml. The paste-like implant that had been prepared as described above was placed to bridge the L5–6 interspinous processes on the interspinous ligament (Fig. 1b). In the control group (BMP0), implants without rhBMP-2 were implanted in the same manner. For the autograft group, autogenous morselized bone harvested from iliac crest was placed. In the sham group, spinous processes were exposed, but no implant was placed. The wounds were then closed with 3-0 absorbable and 3-0 nylon sutures.

Radiological examination of L5–L6 was performed 2, 4, and 8 weeks after surgery. All animals were euthanized by overdose of anesthetics at 8 weeks after surgery, and the

L4–L7 lumbar vertebrae were harvested and processed for further examination. This experimental protocol was approved by The Institutional Committee for Animal Care and Experiments at Osaka City University Medical School.

Manual palpation

Harvested lumbar spines were manually palpated by flexion and extension at the fusion level and solidity was compared with the adjacent level. Each L5–6 segment was graded as showing solid or not solid fusion. Three observers judged the results in terms of fusion. Fusion was graded as solid when the observers unanimously judged fusion to have occurred.

Intradiscal pressure (IDP) measurement

Resin (GC Ostron 2; GC, Aichi, Japan) was used to secure the L5 and L6 endplates. IDP was measured directly with a 1.6-mm-diameter needle-type pressure transducer (FOP-M; FISO Technologies, Quebec, Canada). We inserted an 18-gauge needle from the anterior surface of the annulus into the center of the disc. When the tip of the needle had penetrated the annulus fibrosus, the needle was withdrawn and the transducer tip was placed into the center of the nucleus pulposus of the L5/6 disc. An axial compression force of 0–30 N was applied on the loading board secured to the upper end of a specimen using a materials testing machine (EZ Graph; Shimadzu, Kyoto, Japan). Maximal IDP values were compared with those of control samples.

Bending test

Bending strength of the fusion mass to evaluate solidity of the L5–L6 fusion site was performed by a three-point flexion-bending test using a materials testing machine (EZ Graph; Shimadzu). The L5–6 spinal segment from respective experimental and control groups was fixed to the supporting jig using resin with a 50-mm intersupport distance and bending force was applied by the load cell head at a speed of 1 mm/min. Bending moment at 1.5-mm middle-span deflection was determined from the moment deflection curves (Fig. 2).

Histological examination

The L5–6 segments harvested from respective groups at 8 weeks after surgery were fixed in 4% paraformaldehyde overnight at 4°C, dehydrated in a graded ethanol series, and embedded in plastic resin. Coronal sections of 7 μ m in thickness at the mid-part of the interspinous process were cut with a tungsten blade using a rotary microtome (RM2255; Leica Microsystems, Wetzlar, Germany) and stained with hematoxylin and eosin (HE) after decalcification with 0.5 M ethylenediaminetetraacetic acid for 30 min at room

Fig. 2 Schematic presentation of three-point flexion bending test model. Schematic of a specimen at the L5–L6 intervertebral level as seen from the *lateral view*. *SP* spinous process

temperature or stained with von Kossa without decalcification and observed under light microscopy (BZ-9000; Keyence, Osaka, Japan) to examine for bony fusion by newly formed bone.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation for each group. Comparisons of biomechanical testing and IDP measurement of spines in each group were made using one-way analysis of variance. The Bonferroni/Dunn test was performed to determine significant differences between groups. Values of $P < 0.05$ were considered statistically significant for all tests.

Results

Radiological evaluation

Radiographic images taken immediately after surgery revealed a mass of β -TCP fine particles at the operated site. At 8 weeks after surgery, radiographic images in all animals of the BMP30, BMP60, and BMP120 groups were changed to homogenous calcified shadows connecting the spinous processes. Radiographic images in animals of the BMP0 group revealed remnant β -TCP particles without a homogeneous calcified mass (Fig. 3). No cases showed heterotopic ossification in the spinal canal.

Manual palpation

In all L5–6 spinal segments harvested from the BMP60 and BMP120 groups, bony hard masses bridging the interspinous process were palpable, and passive motion between

vertebrae was significantly restricted when compared to that in the sham and BMP0 groups. Four of ten samples from the BMP30 group and two of ten samples from the autogenous bone group were evaluated as showing solid fusion. In contrast, no samples from the BMP0 or sham groups achieved solid fusion (Table 2).

IDP measurement

IDP at the L5–6 level was significantly lower in the BMP60 and BMP120 groups than in the sham, BMP0, BMP30, and autogenous bone groups (Fig. 4). Reduced IDP in the BMP60 group was significantly reduced by a mean of 34.9% when compared with IDP in the sham group. There are no dose effects between BMP30 and BMP60.

Bending test

Results from bending strength measurements in each experimental group are shown in Fig. 5. Bending moment at 1.5-mm middle span deflection was significantly greater in the BMP60 and BMP120 groups than in the sham, BMP0, BMP30 or autogenous bone groups. The BMP30 group showed significantly greater bending moment than the sham and BMP0 groups. There are no dose effects between BMP30 and BMP60.

Histology

Coronal sections of the L5–6 interspinous fusion mass in animals that received rhBMP-2 (60 or 120 μ g) showed new bone bridging the spinous processes with characteristics of woven bone with hematopoietic marrow. In animals that did not receive rhBMP-2 (BMP0), only fibrous tissue and remaining β -TCP without new bone were observed (Fig. 6). No cases showed heterotopic ossification in the spinal canal or robust inflammation.

Discussion

To manufacture an osteoinductive artificial bone graft substitute, cytokines retaining osteoinductive activity (e.g., BMPs) have been combined with collagen and used to obtain spinal fusion in experimental animals or in human cases [1, 4, 5, 17, 25, 26, 35, 36]. This experimental study was designed to achieve solid posterior interspinous lumbar fusion more effectively and less invasively than before with proper use of rhBMP-2 in combination with synthetic carrier materials.

BMPs are biologically active molecules capable of eliciting new bone formation. In combination with

Fig. 3 Representative lateral radiographs and gross appearance of L5–L6 spine in the BMP0 and BMP60 groups. In the BMP0 group: **a** Immediately after surgery; **b** at 4 weeks; and **c, d** at 8 weeks. In the BMP60 group: **e** Immediately after surgery; **f** at 4 weeks; and **g, h** at

8 weeks. A clear fusion mass (*arrowhead*) is seen between the L5–L6 spinous processes at 8 weeks in the BMP60 sample. *Arrows* show spinous processes

biomaterials, these proteins can be used in clinical settings as bone-graft substitutes to promote bone repair. Collagen from animal sources has previously been the preferred carrier material in animal experiments. However, problems remain with the use of animal-derived collagen, including the risk of antibody generation or disease transmission and lack of mechanical strength of the carrier collagen [3, 8, 10]. To overcome these problems, synthetic biodegradable polymers have recently been tested as a delivery vehicle for osteoinductive agents. Earlier studies performed in our laboratory found that the PLA-PEG block copolymer with plastic properties works effectively as a BMP delivery system [33, 34]. We then attempted to development a new biodegradable bone-inducing material by adding rhBMP-2 to porous β -TCP granules using a PLA-PEG polymer delivery system. The purpose of adding porous β -TCP

Table 2 Fusion rate by manual palpation

Group	Solid fusion
Sham	0/6
BMP 0	0/6
BMP 30	4/10
BMP 60	10/10
BMP 120	10/10
Autogenous bone	2/10

powder was to increase the plasticity of the polymer for ease of handling, increase induced bone yields by volume expansion of the rhBMP-2-containing polymer implants and subsequently improve performance of the rhBMP-2. This new bone-inducing biodegradable porous β -TCP might provide effective artificial bone-graft substitutes

Fig. 4 Operative level IDP under axial compressive loading (-30 N) for a specimen from each group. These results indicate that IDP is significantly lower in BMP60 and BMP120 groups than in sham, BMP0, BMP30, and autogenous bone groups. *Significant difference compared to sham, BMP0, BMP30, and autogenous bone groups. Values represent mean $\pm$ SD

Fig. 5 Results of three-point flexion bending tests. These results indicate significantly stiffer interspinous process fusion in BMP60 and BMP120 groups than in sham, BMP0, BMP30, and autogenous bone groups. *Significant difference compared to sham, BMP0, BMP30, and autogenous bone groups. **Significant difference compared to sham and BMP0 groups. Values represent mean $\pm$ SD

with osteoinductive capacity and biodegradable properties [20, 26]. At the same time, reducing the cost of production for rhBMP-2 is important if clinical use is to become widespread. Currently, rhBMPs are produced using BMP gene-transfected mammalian cell (CHO) cultures [16, 40]. However, production costs are very high due to the low performance efficiency of current technologies for purifying recombinant BMPs produced by mammalian cells. One possible solution to this problem is high-efficiency, low-cost production of monomer rhBMPs in BMP-gene-transfected *E. coli* and conversion of the monomer rhBMPs to dimeric rhBMP using ex vivo biochemical processing. The ectopic induction of new bone formation by dimeric E-BMP-2 has already been reported elsewhere [22, 32]. Recent studies in our laboratory found that characteristics of

E-BMP-2 are satisfactory for clinical use to promote bone healing in place of the currently available BMP-2 produced by BMP-2-expressing CHO cells [12, 43]. We therefore consider that achievement of solid interspinous process fusion using this new biodegradable bone-inducing material could address biomechanical weakness in spinal disorders as a less-invasive option without bone grafting.

The carrier material for BMP-2, a composite of PLA-PEG hydrogel and β -TCP powder, was developed by our group and its efficacy as a BMP-2 carrier has been confirmed in several experimental animal models of various bone defects and spinal fusions [15, 26]. With the use of equivalent amounts of carrier material, the BMP-2-induced new bone mass at the interspinous process region appeared to depend on the BMP-2 dose, and the optimal dose for consistent, solid posterior fusion was 60–120 μ g of BMP-2 in 600 mg of carrier for this rabbit model. However, responsiveness to BMP-2 is known to vary widely depending on the animal species, with high responsiveness in rodents and lower responsiveness in higher mammals [21, 39]. Much higher doses of BMP-2 in addition to a larger amount of carrier material are thus likely to be essential in achieving solid posterior spinal fusion for non-human primates and humans. Optimal doses in a clinical setting should be set based on results for experimental posterior spinal fusion with BMP-2 and synthetic carrier materials in large animals or primates and ultimately from future clinical trials.

This new and simple technology to obtain solid posterior spinal fusion in a short period might present a potential solution to the disadvantages of current techniques for spinal fusion of posterior interspinous processes. With regard to mechanical strength of the fusion mass, significant solidity was noted at 8 weeks after surgery, but this study could not elucidate whether the mechanical strength of the fusion mass is subsequently maintained or remodeled to a stronger bone mass under mechanical stress. IDP after the completion of posterior fusion was reduced to around 65% of the original intact value, meaning that elasticity of the disc was partially retained, as reported previously for posterolateral fusion (PLF) [31]. The remaining elasticity of the disc might reduce the risk of degeneration in discs adjacent to the fused segment, which is often encountered after inter-body fusion [28, 29, 38].

We note several limitations. The first drawback of this study was the use of normal young rabbits with intact spines. If we had used aged rabbits or rabbit with disc degeneration, the results might have differed and consistent solid fusion might not have been obtained using the present methods. The possibility remains that a higher BMP-2 dose might have been required to achieve solid posterior spinal fusion. Another experimental model with segmental instability in the lumbar spine should be used in the next series of

Fig. 6 Histology of coronal sections of L5–6 interspinous process regions. **a** BMP0, **b** BMP30, **c** BMP60, **d** BMP120 (hematoxylin and eosin; magnification $\times 2$). **e** BMP0, **f** BMP30, **g** BMP60, **h** BMP120 (von Kossa and van Gieson; magnification $\times 2$). Newly formed bone

mass bridging spinous processes (SP) in BMP60 and BMP120 groups indicate complete bony fusion of the spinous processes. The BMP0 group showed no new bone formation

studies to clarify the efficacy of BMP-2-retaining bone graft substitute for spinal fusion. Second, we did not compare the interspinous process fusion model with a PLF model in terms of the effective dose of E-BMP-2. Our previous study reported that the effective dose of E-BMP-2 for a rabbit PLF model was 50 μ g [12]. In the present preliminary study, we were able to achieve solid interspinous process fusion using E-BMP-2, but the differences in effective dose for these two models remain unclear. Future studies should evaluate the safety, advantages, invasiveness, and cost-effectiveness of this new approach compared with a PLF model. Third, we have reported the release of BMP-2 in other experimental models, but did not assess the *in vitro* release of BMP-2 in this model of interspinous process fusion. Subsequent serial experimental studies are necessary to develop new, simple, safe, and efficacious methods for spinal fusion before considering application to future clinical practice.

Conclusion

Interspinous process fusion was successfully achieved within 8 weeks in a rabbit model with synthetic bone graft substituting materials (PLA-PEG, rhBMP-2, and β -TCP). The fusion rate with this method was superior to that with autogenous bone grafting. This new method might potentially contribute to the development of simple and safe

surgery to stabilize unstable segments of the spine, although extensive studies are necessary before practical use in spine surgery.

Acknowledgments This research was supported by a Grand-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Project Grant No. 20890196). The authors also wish to thank Ms. K. Hata for technical assistance.

Conflict of interest None.

References

1. Akamaru T, Suh D, Boden SD, Kim HS, Minamide A, Louis-Ugbo J (2003) Simple carrier matrix modifications can enhance delivery of recombinant human bone morphogenetic protein-2 for posterolateral spine fusion. *Spine* 28:429–434
2. Albee FH (1913) An experimental study of bone growth and the spinal bone transplant. *JAMA* 60:1044–1049
3. Bach FH, Fishman JA, Daniels N, Proimos J, Anderson B, Carpenter CB, Forrow L, Robson SC, Fineberg HV (1998) Uncertainty in xenotransplantation: individual benefit versus collective risk. *Nat Med* 4:141–144
4. Boden S, Schimandle J, Hutton W (1995) An experimental lumbar intertransverse process spinal fusion model. Radiographic, histologic, and biomechanical healing characteristics. *Spine (Phila Pa 1976)* 20:412–420
5. Boden SD, Kang J, Sandhu H, Heller JG (2002) Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: a prospective, randomized

- clinical pilot trial: 2002 Volvo Award in clinical studies. *Spine* 27:2662–2673
6. Bono CM, Vaccaro AR (2007) Interspinous process devices in the lumbar spine. *J Spinal Disord Techn* 20:255–261
 7. Bostman O, Myllynen P, Riska EB (1984) Posterior spinal fusion using internal fixation with the Daab plate. *Acta Orthopaedica Scandinavica* 55:310–314
 8. Butler D (1998) Last chance to stop and think on risks of xenotransplants. *Nature* 391:320–324
 9. Cobey MC (1971) The value of the Wilson plate in spinal fusion. *Clinical Orthop Relat Res* 76:138–140
 10. DeLustro F, Dasch J, Keefe J, Ellingsworth L (1990) Immune responses to allogeneic and xenogeneic implants of collagen and collagen derivatives. *Clinical Orthop Relat Res* 260:263–279
 11. DePalma AF, Rothman RH (1968) The nature of pseudarthrosis. *Clinical Orthop Relat Res* 59:113–118
 12. Dohzono S, Imai Y, Nakamura H, Wakitani S, Takaoka K (2009) Successful spinal fusion by *E. coli*-derived BMP-2-adsorbed porous beta-TCP granules: a pilot study. *Clinical Orthop Relat Res* 467:3206–3212
 13. Drummond D, Guadagni J, Keene JS, Breed A, Narechania R (1984) Interspinous process segmental spinal instrumentation. *J Pediatr Orthop* 4:397–404
 14. Heller KD, Prescher A, Schneider T, Block FR, Forst R (1998) Stability of different wiring techniques in segmental spinal instrumentation. An experimental study. *Arch Orthop Trauma Surg* 117:96–99
 15. Hoshino M, Egi T, Terai H, Namikawa T, Takaoka K (2007) Regenerative repair of long intercalated rib defects using porous cylinders of beta-tricalcium phosphate: an experimental study in a canine model. *Plast Reconstr Surg* 119:1431–1439
 16. Israel DI, Nove J, Kerns KM, Kaufman RJ, Rosen V, Cox KA, Wozney JM (1996) Heterodimeric bone morphogenetic proteins show enhanced activity in vitro and in vivo. *Growth factors (Chur, Switzerland)* 13:291–300
 17. Johnsson R, Stromqvist B, Aspenberg P (2002) Randomized radiostereometric study comparing osteogenic protein-1 (BMP-7) and autograft bone in human noninstrumented posterolateral lumbar fusion: 2002 Volvo Award in clinical studies. *Spine* 27:2654–2661
 18. Jutte PC, Castelein RM (2002) Complications of pedicle screws in lumbar and lumbosacral fusions in 105 consecutive primary operations. *Eur Spine J* 11:594–598
 19. Kasai Y, Inaba T, Akeda K, Uchida A (2008) Tadpole system as new lumbar spinal instrumentation. *J Orthop Surg Res* 3:41
 20. Kato M, Namikawa T, Terai H, Hoshino M, Miyamoto S, Takaoka K (2006) Ectopic bone formation in mice associated with a lactic acid/dioxanone/ethylene glycol copolymer-tricalcium phosphate composite with added recombinant human bone morphogenetic protein-2. *Biomaterials* 27:3927–3933
 21. Kato M, Toyoda H, Namikawa T, Hoshino M, Terai H, Miyamoto S, Takaoka K (2006) Optimized use of a biodegradable polymer as a carrier material for the local delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2). *Biomaterials* 27:2035–2041
 22. Kubler NR, Reuther JF, Faller G, Kirchner T, Ruppert R, Sebald W (1998) Inductive properties of recombinant human BMP-2 produced in a bacterial expression system. *Int J Oral Maxillofac Surg* 27:305–309
 23. Lindholm TS, Nilsson OS, Lindholm TC (1982) Extraskelatal and intraskelatal new bone formation induced by demineralized bone matrix combined with bone marrow cells. *Clin Orthop Relat Res* 171:251–255
 24. Matsushita N, Terai H, Okada T, Nozaki K, Inoue H, Miyamoto S, Takaoka K (2004) A new bone-inducing biodegradable porous beta-tricalcium phosphate. *J Biomed Mater Res* 70:450–458
 25. Minamide A, Kawakami M, Hashizume H, Sakata R, Tamaki T (2001) Evaluation of carriers of bone morphogenetic protein for spinal fusion. *Spine* 26:933–939
 26. Namikawa T, Terai H, Suzuki E, Hoshino M, Toyoda H, Nakamura H, Miyamoto S, Takahashi N, Ninomiya T, Takaoka K (2005) Experimental spinal fusion with recombinant human bone morphogenetic protein-2 delivered by a synthetic polymer and beta-tricalcium phosphate in a rabbit model. *Spine* 30:1717–1722
 27. Oikarinen J (1982) Experimental spinal fusion with decalcified bone matrix and deep-frozen allogeneic bone in rabbits. *Clin Orthop Relat Res* 162:210–218
 28. Okuda S, Miyauchi A, Oda T, Haku T, Yamamoto T, Iwasaki M (2006) Surgical complications of posterior lumbar interbody fusion with total facetectomy in 251 patients. *J Neurosurg Spine* 4:304–309. doi:10.3171/spi.2006.4.4.304
 29. Park P, Garton HJ, Gala VC, Hoff JT, McGillicuddy JE (2004) Adjacent segment disease after lumbar or lumbosacral fusion: review of the literature. *Spine* 29:1938–1944
 30. Reeg SE (2001) A review of comorbidities and spinal surgery. *Clin Orthop Relat Res* 384:101–109
 31. Rolander SD (1966) Motion of the lumbar spine with special reference to the stabilizing effect of posterior fusion. An experimental study on autopsy specimens. *Acta orthopaedica Scandinavica* 90(Suppl):91–144
 32. Ruppert R, Hoffmann E, Sebald W (1996) Human bone morphogenetic protein 2 contains a heparin-binding site which modifies its biological activity. *Eur J Biochem/FEBS* 237:295–302
 33. Saito N, Okada T, Horiuchi H, Murakami N, Takahashi J, Nawata M, Ota H, Nozaki K, Takaoka K (2001) A biodegradable polymer as a cytokine delivery system for inducing bone formation. *Nat Biotechnol* 19:332–335
 34. Saito N, Takaoka K (2003) New synthetic biodegradable polymers as BMP carriers for bone tissue engineering. *Biomaterials* 24:2287–2293
 35. Sandhu HS, Kanim LE, Kabo JM, Toth JM, Zeegen EN, Liu D, Delamarter RB, Dawson EG (1996) Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion. *Spine* 21:2115–2122
 36. Schimandle J, Boden S, Hutton W (1995) Experimental spinal fusion with recombinant human bone morphogenetic protein-2. *Spine (Phila Pa 1976)* 20:1326–1337
 37. Steinmann JC, Herkowitz HN (1992) Pseudarthrosis of the spine. *Clin Orthop Relat Res* 284:80–90
 38. Swanson KE, Lindsey DP, Hsu KY, Zucherman JF, Yerby SA (2003) The effects of an interspinous implant on intervertebral disc pressures. *Spine* 28:26–32
 39. Valentin-Opran A, Wozney J, Csimma C, Lilly L, Riedel GE (2002) Clinical evaluation of recombinant human bone morphogenetic protein-2. *Clin Orthop Relat Res* 395:110–120
 40. Wang EA, Rosen V, D'Alessandro JS, Bauduy M, Cordes P, Harada T, Israel DI, Hewick RM, Kerns KM, LaPan P et al (1990) Recombinant human bone morphogenetic protein induces bone formation. *Proc Natl Acad Sci USA* 87:2220–2224
 41. Wang JC, Haid RW Jr, Miller JS, Robinson JC (2006) Comparison of CD HORIZON SPIRE spinous process plate stabilization and pedicle screw fixation after anterior lumbar interbody fusion. Invited submission from the Joint Section Meeting on Disorders of the Spine and Peripheral Nerves, March 2005. *J Neurosurg* 4:132–136
 42. Weigel U, Meyer M, Sebald W (1989) Mutant proteins of human interleukin 2. Renaturation yield, proliferative activity and receptor binding. *Eur J Biochem/FEBS* 180:295–300
 43. Yano K, Hoshino M, Ohta Y, Manaka T, Naka Y, Imai Y, Sebald W, Takaoka K (2009) Osteoinductive capacity and heat stability of recombinant human bone morphogenetic protein-2 produced by *Escherichia coli* and dimerized by biochemical processing. *J Bone Mineral Metab* 27:355–363