

# Optimal Intermittent Administration Interval of parathyroid hormone 1-34 for Bone Morphogenetic Protein-Induced Bone Formation in a Rat Spinal Fusion Model

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## Research article

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# Abstract

**Summary of Background Data.** Both bone morphogenetic protein 2 (BMP-2) and parathyroid hormone (PTH) have been used to enhance bone healing. There is still no established opinion as to the optimum dose and administration method. We investigated to clarify the optimal dose and administration method for the combination of BMP-2 and PTH in a rat spinal fusion model.

**Methods.** A total of 52 rats were performed spinal fusion and were divided into four groups. Group I (n = 10) was implanted with control carrier. Group II (n = 14) was implanted with carrier containing 3 µg of recombinant human BMP-2 (rhBMP-2). Group III (n = 14) was implanted with carrier containing 3 µg of rhBMP-2, followed by injections of PTH three times a week (total, 180µg/kg/week). Group IV (n = 14) was implanted with carrier containing 3 µg of rhBMP-2, followed by injections of PTH six times a week (total, 180µg/kg/week). The rats were euthanized after 8 weeks and their spines were explanted and assessed by manual palpation, radiographs, and high-resolution micro computerized tomography (micro-CT); the spines were also subjected to histological analysis. Serum markers of bone metabolism were also analyzed.

**Results.** Manual palpation tests showed that the fusion rates of Groups III (28.6%) and IV (35.7%) were considerably higher than those of Group I. Groups III and IV had higher radiographic scores compared to Group I. Micro-CT analysis revealed enhanced bone marrow density expressed as bone volume/tissue volume in Group IV ( 54.20 ± 2.49%) versus Group I ( 38.56 ± 7.78%). Serum makers analysis revealed that Group IV had higher osteocalcin and lower tartrate-resistant acid phosphatase-5b than those of Group III. A histological analysis indicated that Group VI had enhanced remodeling.

**Conclusions.** The combination of rhBMP-2 and PTH enhanced bone fusion and remodeling of newly formed bone in a rat spinal fusion model. More frequent administration may be superior in terms of bone fusion and bone metabolism.

## Introduction

There are some reports of bone fusion caused by the combination of spinal arthrodesis and various drugs in the past. Spinal arthrodesis is a fundamental treatment option for spinal pathologies and one of the most common spinal procedures, with more than 20,000 surgeries performed in the United States each year <sup>1)</sup>. In this procedure, bone grafts are used to restore mechanical stability to the affected spinal segment by providing bridging bone between vertebrae. Because successful bone fusion between unstable spinal segments leads to pain relief and neurologic recovery, the efficacy of this procedure has gained wide acceptance, and the number of these types of surgery has increased annually with the increase in the aged population <sup>2-5)</sup>.

Bone morphologic proteins (BMPs) are members of the transforming growth factor-β superfamily <sup>6)</sup>, and are powerful osteoinductive molecules. BMPs also are considered to promote new osteoclast formation since they stimulate the production of receptor activator of nuclear factor kappa-β ligand (RANKL)

osteoblasts and help ensure mature osteoclast survival; therefore, BMPs participate in bone matrix resorption<sup>7-8</sup>). The osteoinductive effects of recombinant human BMP-2 (rhBMP-2) for spinal fusion have been shown in animal models and clinical trials<sup>9-13</sup>). Although BMPs are approved for clinical use, clinical trial results have shown that high doses are required to induce adequate bone fusion because of the following reasons: (1) solubility of the molecules, (2) easy diffusion of the molecules away from the fusion site, and (3) in vivo inactivation<sup>14</sup>). In addition, BMPs are expensive; therefore, their usefulness may be limited by their expense. As a result, a number of strategies are being developed to provide a safer, less expensive, and more efficacious spinal fusion using rhBMP-2.

Human parathyroid hormone (PTH) 1-34 is an anabolic drug, and its efficacy as an osteoporosis drug has been widely verified through experimental and clinical studies<sup>15-18</sup>). Although PTH could have bone resorption effects with continuous administration, it can accelerate bone formation with intermittent administration. PTH can also enhance fracture healing not only in ovariectomized rats but also in intact rats<sup>19,20</sup>). The clinical use of PTH to accelerate fracture healing has been attempted, and the combination of PTH and BMPs has been shown favor spinal fusion<sup>21</sup>). But the optimal intermittent administration interval of PTH has not been revealed.

We previously reported that the synergic effect of rhBMP-2 and PTH administered systemically as a single dose at the optimal time was efficacious for fracture repair, and significantly enhanced bone fusion in a rat femoral fracture model<sup>22</sup>). The purpose of this study was to clarify the optimal dosing interval and administration method for the combination of rhBMP-2 and PTH in a rat spinal fusion model.

## Material And Method

### *Preparation of matrices*

CollaCote<sup>®</sup> (Zimmer/dental) is a biodegradable collagen scaffolding used for cellular attachment. Although a collagen sponge has been used clinically as a carrier for rhBMP-2<sup>23,24</sup>), CollaCote<sup>®</sup> has an allograft matrix possess hemostatic function that facilitates early clot formation and wound stabilization. The final concentration of rhBMP-2 (Peptotech, Rocky Hill, NJ) was dissolved in phosphatebuffered saline (PBS) buffer (pH 7.5) and applied to CollaCote<sup>®</sup>. CollaCote<sup>®</sup> was cut with a scalpel into 5 × 20-mm strips and placed with rhBMP-2 in an Eppendorf tube that was left overnight at 4 °C prior to implantation. Similarly, 100 µL of rhBMP-2-free PBS was dropped in CollaCote<sup>®</sup> to obtain rhBMP-2-free CollaCote<sup>®</sup>.

### *Study groups*

A total of 52 male Sprague–Dawley rats (8–10 weeks old; CLEA Japan, Inc., Tokyo, Japan) were divided into four groups. Group I (n = 10) included animals that were implanted with control carrier alone. Group II (n = 14) included animals that were implanted with carrier containing 3µg of rhBMP-2. Group III (n = 14) included animals that were implanted with carrier containing 3µg of rhBMP-2, followed by injections of

PTH (Teribone; Asahi Kasei Pharma, Tokyo, Japan) (60µg/kg) three times a week (total, 180µg/kg/week). Group IV (n = 14) included animals that were implanted with carrier containing 3 µg of rhBMP-2, followed by injections of PTH (30µg/kg) six times a week (total, 180µg/kg/week). Rats in groups III and IV were subcutaneously injected with PTH beginning 1 week after surgery. The injections were continued until immediately before the rats were euthanized.

### ***Surgical Technique for Constructing the L4-L5 Posterolateral Spinal Fusion Model***

All animal studies were approved by the Oita University animal research committee, and experiments conformed to all guidelines and regulations for the protection of welfare of animals (protocol No. 1624002).

The rats were anesthetized by an intraperitoneal injection containing 0.3–0.4 ml of 0.15 mg/kg medetomidine + 2 mg/kg midazolam + 2.5 mg/kg butorphanol. Disinfect the surgical site with 0.05% Chlorhexidine Gluconate. A posterior midline incision was made on the skin. Next, two separate paramedian incisions were made at 3 mm from the midline in the lumbar fascia and the transverse processes were exposed. The transverse processes of L4 and L5 were decorticated using a low-speed burr. Subsequently, CollaCote with or without rhBMP-2 was implanted on each side. The fascial and skin incisions were closed with a 3–0 absorbable suture. After surgery, the rats were given Gentamicin by intramuscular injection for three successive days. Immediately following surgery and on subsequent days, the rodents received analgesics (buprenorphine subcutaneously and paracetamol). The rodents were housed in separate cages and fed food and water ad libitum and their condition was monitored on a daily basis. The rats were humanly euthanized eight weeks post operatively.

### ***Manual assessment of fusion***

Eight weeks post-implantation, the explanted spines were manually tested for intersegmental motion by three blinded independent observers<sup>25-31</sup>). The explanted lumbar spine was palpated gently and the lateral side bending motion at the L4-L5 level was compared with the motion at the adjacent levels above (L3-L4) and below (L5-L6). The absence of motion was considered as successful fusion. Any motion detected between the transverse processes was considered as a failure of fusion. The spine was designated as “not fused” if any of the three observers graded the spine as not fused. The spines were scored as either fused or not on both the right and left sides and the fusion rate was then calculated.

### ***Radiographic Analysis***

The explanted spines obtained at the 8-week time point were photographed using a Softex X-ray apparatus (Softex CSM-2; Softex, Tokyo, Japan) employing HS Fuji Softex film (Fuji Film, Tokyo, Japan) at 45 cm with 30 kV and 15 mA for 20 s. Fusion between the L4 and L5 transverse processes in each rat was recorded as a percentage of the total area between the L4 and L5 that was filled with new bone<sup>25</sup>). Three blinded independent observers scored the bone formation in each rat using a 5-point scale: 0 = no bone formation; 1 = bone filling in less than 25% of the area; 2 = bone filling in 25–50% of the area; 3 =

bone filling in 50–75% of the area; and 4 = bone filling in 75–100% of the area. The spines were scored on both the right and left sides.

### ***Micro-CT Analysis***

The spines were scanned by micro-CT using SkyScan 1172 (Bruker microCT, Kontich, Belgium) with a voxel size of 20  $\mu$ m. The data were collected at 100 kV and 100 mA and reconstructed using the cone-beam algorithm. Each spine was set on the object stage and sample scanning was performed over a 180° rotation with an exposure time of 105 ms. A cylindrical volume of interest with a diameter of 20 mm and a height of 27 mm, which displayed the micro-structure of the rat vertebra as comprised of cortical and cancellous bone, was selected. Data analysis of the area from the top of the L4 transverse processes to the bottom of the L5 transverse processes, including the vertebrae, was performed using the CT Analyzer software (Bruker microCT). The spines were analyzed on both the right and left sides. In the bone mineral density (BMD), tissue volume (TV), bone volume (BV), trabecular thickness (Tb. Th), trabecular spacing (Tb. Sp), and bone volume fraction (BV/TV, %) were measured.

### ***Analysis of Serum Markers of Bone Metabolism***

Just prior to euthanization of the animals, blood samples were collected and stored at -80°C until the serum markers of bone metabolism were analyzed. Serum markers of bone metabolism were analyzed with use of an enzymelinked immunosorbent assay specific for osteocalcin (Osteocalcin High Sensitive EIA kit [rat]; Takara Bio, Shiga, Japan) and tartrate-resistant acid phosphatase-5b (TRACP5b) (RatTRAP Assay; Immunodiagnostic Systems), according to the manufacturer's instructions.

### ***Histological Analysis***

Eight weeks post implantation, the spines were dissected and the specimens were fixed in 40% ethanol, decalcified using standard 10% decalcifying solution HCl (Cal-Ex; Fischer Scientific, Fairlawn, NJ), washed with running tap water, and then transferred to 75% ethanol. Serial sagittal sections near the transverse processes were cut carefully at the level of the transverse process on both the right and left sides. The specimens were embedded in wax for sectioning. Sagittal sections (5 mm) were cut from the paraffin blocks using a microtome (LS-113; DAIWA-KOKI, Saitama, Japan). The sections were stained with hematoxylin and eosin for basic morphology. Three blinded independent observers scored histological bone formation. Histological fusion was defined as bony trabeculae bridging from one transverse process to the next<sup>25</sup>. Fusion masses were assessed and the extent of new bone formation was scored using the following scoring criteria: 1 = fibrocartilage tissue filling less than 25% of the gap area; 2 = fibrocartilage tissue filling 25–50% of the gap area; 3 = fibrocartilage and bone tissue filling 75–99% of the gap area; 4 = bridged with bone tissue, but the fusion masses are comprised of thin trabecular bone; and 5 = completely bridged with abundant mature bone tissue. The spines were scored on both the right and left sides.

### ***Statistical methods***

The computer program Statistical Package for the Social Sciences (SPSS) (V13; IBM Corporation, Armonk, NY) was used for statistical analysis. Analysis of variance was used for statistical analysis. p values <0.05 were considered significant. A kappa statistic was calculated as a measure of the interobserver reliability of the three independent blinded observers. The kappa statistic corrects the observed agreement for possible chance agreement among observers. Agreement was rated as follows: poor,  $k=0-0.20$ ; fair,  $k=0.21-0.40$ ; moderate,  $k=0.41-0.60$ ; substantial,  $k=0.61-0.80$ ; and excellent,  $k>0.81$ . A value of one indicated absolute agreement, whereas a value of 0 indicated agreement no better than chance.

## Results

No abnormal behavior or neurological deficits were noted in any of the 52 rats before or after the surgical procedure or at the time of euthanasia. No adverse events have been identified in the groups receiving BMP-2, PTH, and in the control group.

### *Manual Palpation*

Table 1 shows the proportion of subjects in each Group that achieved fusion according to the three independent evaluators. Consistent agreement ( $\kappa = 0.864$ ) was noted among the three independent observers who performed manual palpation.

8 segments in Group III ( $n=14$ , segments=28) were assessed as fused (fusion rate, 28.6%) and 10 segments in Group IV ( $n=14$ , segments=28) exhibited fusion (fusion rate, 35.7%), while 2 segments in Group II ( $n=14$ , segments=28) exhibited fusion (fusion rate, 7.1%). None of the spines in Group I ( $n=10$ , segments=20) were fused (fusion rate, 0%). The subjects in Group III and IV had higher fusion rates than in the Group I and II. There was no significant difference between the manual assessment scores of Groups I and II, whereas significantly higher fusion rates were observed in Group III and IV than in Groups I ( $p<0.05$ ). No significant difference was found between groups III and IV.

### *Radiographic analysis*

Radiographs of the spines were obtained at 8 weeks. Consistent agreement ( $\kappa = 0.812$ ) was noted among the three independent observers who graded the radiographs. The spines were scored on both the right and left sides. The average evaluation scores for each Group are shown in Table 2, and the representative anteroposterior (AP) radiographs of the representative case in each Group at 8 weeks are shown in Figure 1. At eight weeks postoperatively, Group III and IV showed evidence of bone formation between the L4 and L5 transverse processes and bony bridging was detected. Mineralized callus bridging between the L4 and L5 transverse processes was detected in Group II, although the amount of callus was deemed insufficient. Group I showed no evidence of bone formation. The scores of Groups III and IV were significantly higher than those of Group I ( $p<0.05$ ).

### *Micro-CT analysis*

A computer analysis of the micro-CT images revealed the volume of new bone and the quality of the spinal fusion area. The average micro-CT data based on the histomorphometry of each Group are shown in Tables 3 and 4. The statistical analysis revealed a statistical difference in the variance in the bone volume percentage. The bone volume percentage of Group II was larger than that of Group I ( $p = 0.023$ ). Trabecular thickness ( $p = 0.041$ ) and trabecular spacing ( $p = 0.026$ ) also had statistical significance between Group II and III. Trabecular thickness ( $p = 0.040$ ) had statistical significance between Group II and III.

### ***Serum Markers of Bone Metabolism***

Enzyme-linked immunosorbent assay demonstrated that serum levels of osteocalcin had no significant differences in each Group II, III, and IV compared with Group I. On the other hand, a significant difference was found between Group III and Group IV ( $p < 0.05$ ) (Figure 2). Identically, serum levels of TRACP5b had no significant differences in each Group II, III, and IV compared with Group I. Whereas a significant difference was found between Group III and Group IV ( $p < 0.05$ ) (Figure 3).

### ***Histological analysis***

Histological analysis of Group I showed a paucity of new bone formation and no evidence of fusion (Figure 4A, B). These images clearly demonstrate muscle between the transverse processes for both specimens. Occasional evidence of new bone formation was observed, originating either from the decorticated transverse process or normal remodeling. Group II showed distribution of cartilaginous tissue, but there was fibrosis tissue and muscle fiber between the transverse processes and no evidence of bone fusion (Figure 5A, B). Analysis of Group III showed distribution of cartilaginous tissue and immature bone formation, but no woven bone between the transverse processes (Figure 6A, B). Group IV showed new bone formation bridging the transverse processes demonstrating mature osteoid tissue and trabeculae contracts (Figure 7A, B). Histological fusion scores are shown in Table 5. The spines were scored on both the right and left sides. Group III and IV had significantly higher histological scores than the Group I ( $p < 0.05$ ). Consistent agreement ( $k=0.862$ ) was noted among the three independent observers.

## **Discussion**

In the present study, we investigated the optimal intermittent administration interval dosing of PTH using BMPs for spinal fusion in a rat spinal fusion model. Posterolateral lumbar fusion in rats has been well established as an acceptable model for measuring bone growth and manual palpation is the most sensitive and specific method for assessing spinal fusion<sup>25-32</sup>). Although a generous amount of PTH effectively demonstrated fracture healing in rat models<sup>19</sup>), the clinical application for humans must be much larger. A clinical dose of PTH to treat human osteoporosis is 20–40 µg daily in Japan and the USA. A dose of 5 µg/kg/day seemed ineffective for changing the mechanical properties in a rat<sup>20</sup>). Morimoto et al. reported successful spinal fusion with weekly doses of 180 µg/kg/week PTH in a rat model of spinal fusion<sup>33</sup>). Therefore, we adopted 180 µg/kg/week (3 times/week and 6 times/week). Due to the

difference in metabolism between humans and rats, it is assumed that the 6 times weekly administration Group is approximately 2 times weekly administration in human, and the 3 times weekly administration Group is approximately once weekly administration in human <sup>34</sup>).

Recent studies of the interactions between PTH and BMP showed that there are two types of interactions between PTH and rhBMP-2 <sup>35</sup>). The first is a mechanism that occurs through a complex comprising PTH type-1 receptors (PTH1R), low-density lipoprotein receptor-related protein 6 (LRP6) within the canonical Wnt pathway, and BMP antagonists. Endocytosis of the complex suppresses negative feedback of BMP antagonists, thereby stimulating BMP signaling. Through that mechanism, PTH enhances the differentiation of mesenchymal stem cells (MSCs) into osteoblasts. The second is a mechanism that occurs through peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ). Excessive BMP signaling results in downregulation of the Wnt pathway through sclerostin. The downregulated Wnt pathway results in increased PPAR $\gamma$ , which persuades MSCs to differentiate into adipocytes. PTH could activate the Wnt pathway and suppress PPAR $\gamma$ . Morimoto et al. reported synergistic anabolic effects with the concomitant use of intermittent PTH and locally applied rhBMP-2 in a rat model of spinal fusion <sup>33</sup>), in their study, the combination increased fusion rates and improved the quality of the newly formed bone. In the present study, Groups III and IV had significantly better fusion rates than Group I according to the manual palpation tests, and higher radiographic evaluation scores. They also had a better fusion rate than Group II. The micro-CT analysis indicated that Group III and IV had significantly higher BV/TV scores compared to Group I, which indicated increased bone marrow density. Trabecular thickness (Tb. Th) and trabecular number (Tb. N) also showed statistical differences, so PTH has the effect of strengthening the trabecular bone structure, and the effect may be stronger when administered 6 times a week. Although no significant difference was observed, Group IV tended to be superior to Group III.

Earlier studies investigating the long-term daily administration of PTH in ovariectomized rats <sup>36,37</sup>) did not report its effects on bone metabolism markers. However, in a prior study, daily administration of PTH at a dose of 30  $\mu$ g/kg for 12 months led to a 40 % increase in osteocalcin, a bone formation marker, relative to the control Group <sup>38</sup>). In our study, six-times-weekly administration of PTH at 30  $\mu$ g/kg (180  $\mu$ g/kg/week) elicited a similar increase in osteocalcin. We observed decrease in serum TRAP5b following six-times-weekly administration of teriparatide compared with control Group. On the other hand, in the three-times-weekly administration Group, neither osteocalcin nor TRAP5b was significantly different from the control Group. These results suggest that daily administration of PTH increases bone formation and bone resorption, with enhanced bone turnover favoring bone formation, whereas six-times-weekly administration of teriparatide may enhance bone formation with no accompanying increase in bone resorption, so six times a week dosing may be the best.

The results of the histological analysis revealed that Group III and IV had well developed bone trabeculae and bone marrow and mature osteocytes that were not seen in Groups I and II. Group IV also showed an almost normal appearance of the bone marrow compared to Group III. This could be evidence of enhanced remodeling caused by more frequent PTH administration.

Limitations of the present study was that we could not see the bone fusion process because we decided to sacrifice the rats at 8 weeks after surgery, when complete bone fusion of the spine had occurred. Despite this, we chose to use a practical dose of rhBMP-2 and PTH in the clinical setting, which was advantageous to the study. Although we cannot directly apply our findings to humans because the rat has different biological reactions to drugs, we believe that the results of the current study provide further evidence for understanding the effects of these therapeutic agents.

In conclusion, the combination of abundant rhBMP-2 and PTH enhanced bone fusion and remodeling of newly formed bone in a rat spinal fusion model. More frequent administration may be superior in terms of bone formation.

## **Abbreviations**

BMP-2: Bone morphogenetic protein 2; BMPs: Bone morphogenetic proteins; BV: bone volume; LRP6: lipoprotein receptor-related protein 6; PBS: phosphate-buffered saline; PPAR $\gamma$ : peroxisome proliferator-activated receptor gamma; PTH: Parathyroid hormone; PTH1R: PTH type-1 receptors; rhBMPs: recombinant human BMPs; Tb.N: trabecular number; Tb.Th: Trabecular thickness; TV: tissue volume

## **Declarations**

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### **Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

### **Authors' contributions**

TA, SK, TI, and NN carried out the operation. HT conceived of the study design. TA, MM, YT, MK, and HT interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

### **Ethics approval**

This study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the Oita University animal research committee prior to animal experimentation (Oita University institutional Animal Ethics Committee no.1624002).

### **Consent for publication**

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Tables

**Table 1.** Assessment of Spinal Fusion Via Manual Palpation.

Treatment Group		No. Assessed Manually for Fusion	No. Assessed as Fused	Fusion Rate (%)
Group I	Carrier alone	20	0	0
Group II	3µg rhBMP-2	28	2	7.1
Group III	3µg rhBMP-2 + PTH (3 times/week)	28	8	28.6*
Group IV	3µg rhBMP-2 + PTH (6 times/week)	28	10	35.7*

\*p < 0.05 (vs Group I)

**Table 2.** Radiographic Scores at 8 weeks.

Treatment Group		No. studied radiographically	Score at 8 weeks (mean ± SD)
Group I	Carrier alone	20	0.11 ± 1.41
Group II	3µg rhBMP-2	28	0.48 ± 1.55
Group III	3µg rhBMP-2 + PTH (3 times/week)	28	1.75 ± 1.37*
Group IV	3µg rhBMP-2 + PTH (6 times/week)	28	2.11 ± 1.21*

\*p < 0.05 (vs Group I)

**Table 3.1.** Micro-CT Based Histomorphometry of Spines at 8 weeks.

Treatment Group		TV (mm <sup>3</sup> )	BV (mm <sup>3</sup> )	BV/TV (%)
Group I	Carrier alone	123.71 ± 6.88	47.44 ± 8.56	38.56 ± 7.78
Group II	3µg rhBMP-2	119.87 ± 13.30	44.19 ± 9.45	37.05 ± 7.77
Group III	3µg rhBMP-2 + PTH (3 times/week)	114.96 ± 7.45	49.19 ± 8.80	42.97 ± 8.29
Group IV	3µg rhBMP-2 + PTH (6 times/week)	108.13 ± 12.84	58.82 ± 9.68	54.20 ± 2.49*

TV, tissue volume; BV, bone volume; BV/TV, bone volume fraction.

\*p < 0.05 (vs Group I)

**Table 3.2.** Micro-CT based histomorphometry of spines at 8 weeks.

Treatment Group		Tb. Th (mm)	Tb. N (1/mm)	Tb. Sp (1/mm)
Group I	Carrier alone	0.22 ± 0.05	1.70 ± 0.04	0.41 ± 0.11
Group II	3µg rhBMP-2	0.21 ± 0.06	1.81 ± 0.14	0.46 ± 0.13
Group III	3µg rhBMP-2 + PTH (3 times/week)	0.21 ± 0.06	2.11 ± 0.31*	0.38 ± 0.14
Group IV	3µg rhBMP-2 + PTH (6 times/week)	0.30 ± 0.03*	1.84 ± 0.29	0.20 ± 0.05*

Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular spacing.

\*p < 0.05 (vs Group I)

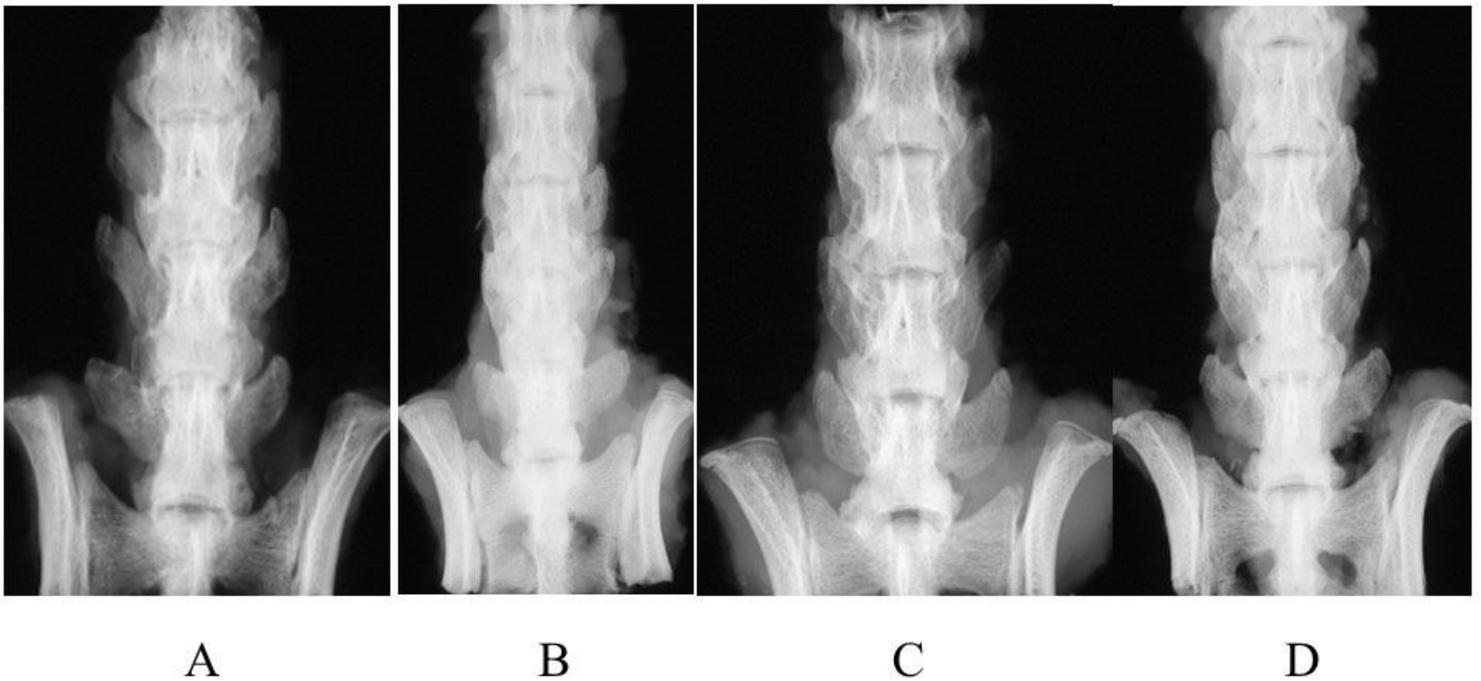
**Table 4.** Histologic fusion score at 8 weeks.

Treatment Group		Score at 8 weeks (mean $\pm$ SD)
Group I	Carrier alone	0.10 $\pm$ 1.02
Group II	3 $\mu$ g rhBMP-2	0.68 $\pm$ 1.33
Group III	3 $\mu$ g rhBMP-2 + PTH (3 times/week)	2.66 $\pm$ 1.21*
Group IV	3 $\mu$ g rhBMP-2 + PTH (6 times/week)	3.15 $\pm$ 1.73*

\*p < 0.05 (vs Group I)

## Figures

Figure 1.



**Figure 1**

Radiographs of rat spines obtained at 8 weeks post posterolateral lumbar fusion surgery. (A) Group I (carrier alone), (B) Group II (carrier containing 3  $\mu$ g rhBMP-2), (C) Group III (carrier containing 3  $\mu$ g rhBMP-

2 + PTH (3 times/week)), and (D) Group IV (carrier containing 3  $\mu$ g rhBMP-2 + PTH (6 times/week)). Group IV showed evidence of bone formation between the L4 and L5 transverse processes and bony bridging was detected. The mineralized callus bridging between the L4 and L5 transverse processes was detected in Group III, although the amount of callus was deemed insufficient. Groups I and II showed no evidence of bone formation.

Figure 2.

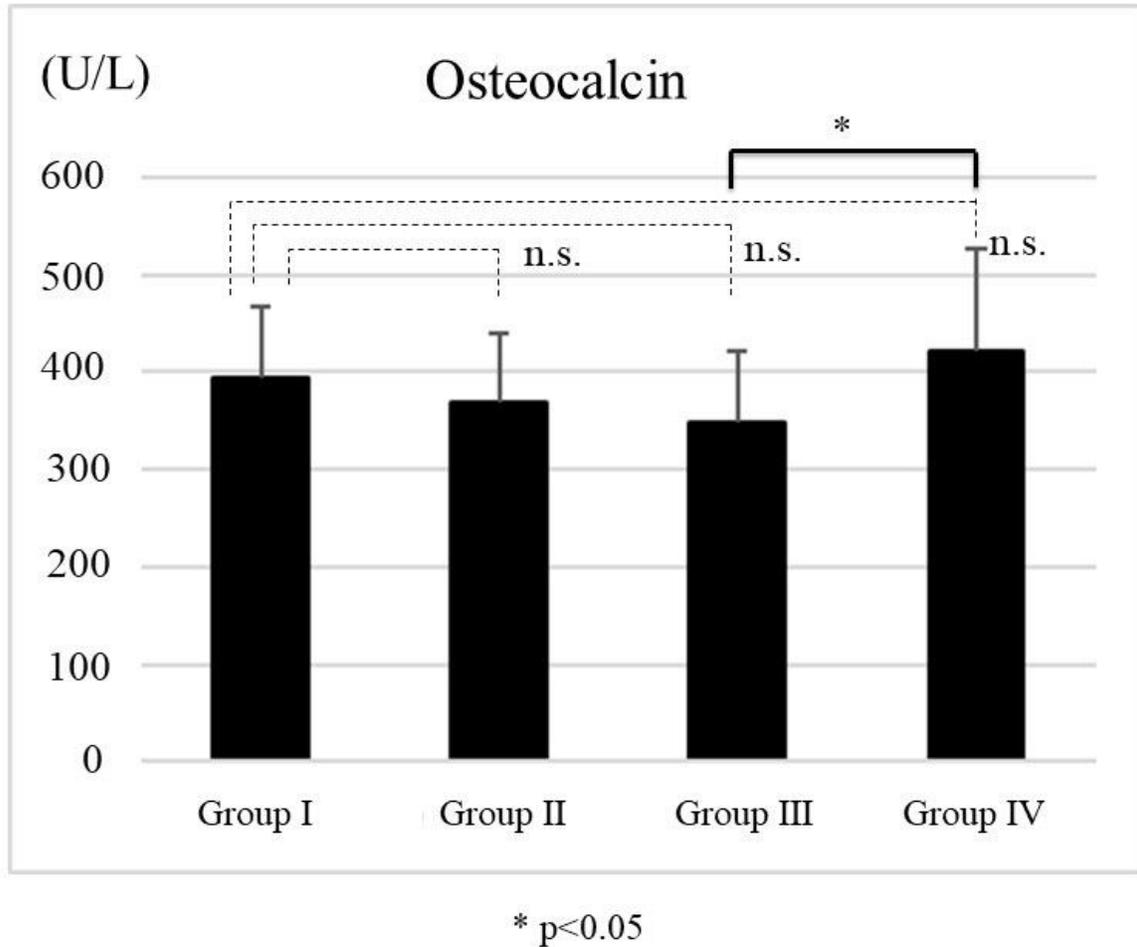


Figure 2

Enzyme-linked immunosorbent assay demonstrated that serum levels of osteocalcin showed no significant differences in Groups II, III, and IV compared with that in Group I. In contrast, a significant difference was observed between Groups III and IV ( $p < 0.05$ ).

Figure 3.

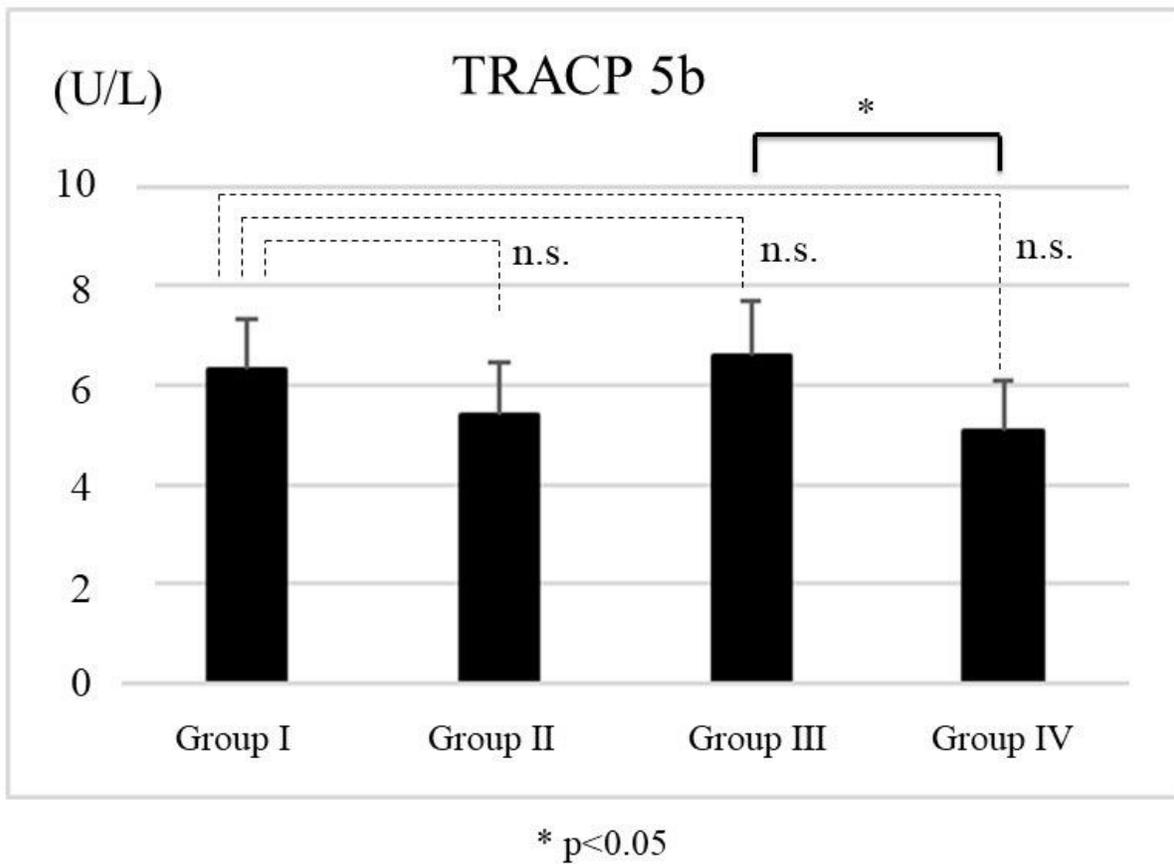
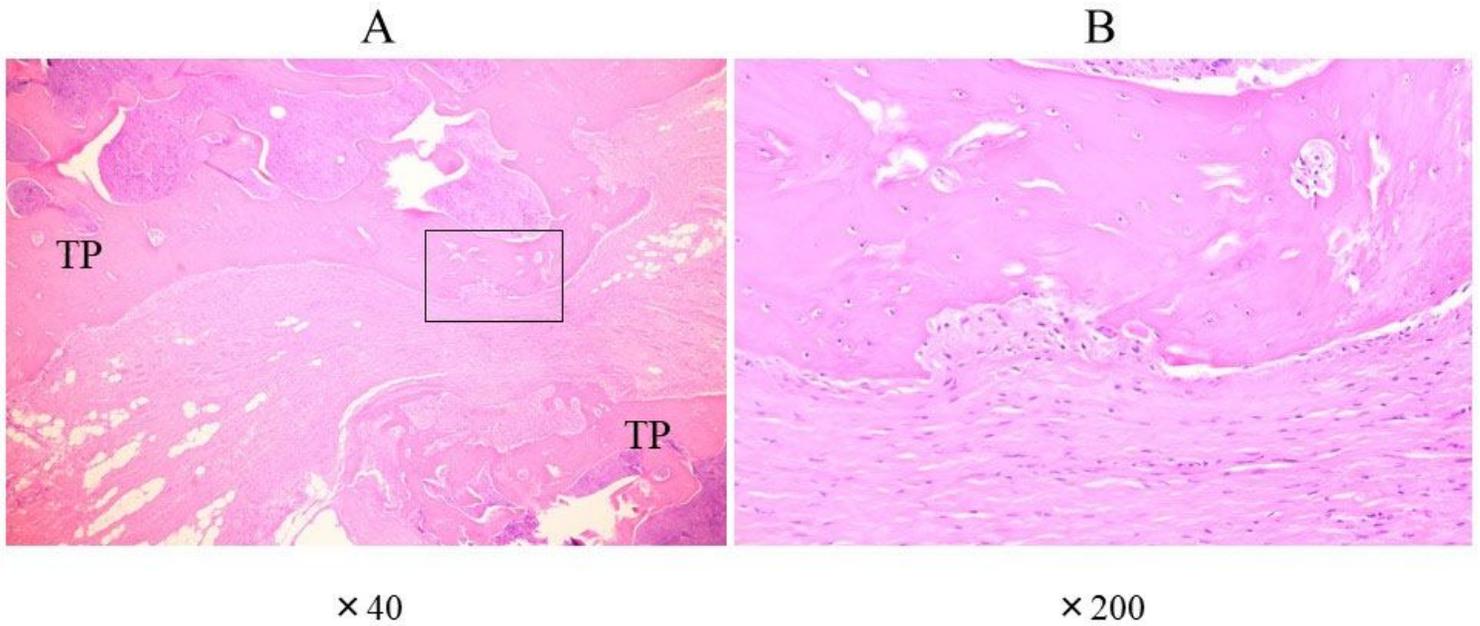


Figure 3

Serum levels of tartrate-resistant acid phosphatase-5b (TRACP5b) showed no significant differences between Groups II, III, and IV compared with that in Group I, whereas a significant difference was found between Groups III and IV ( $p < 0.05$ ).

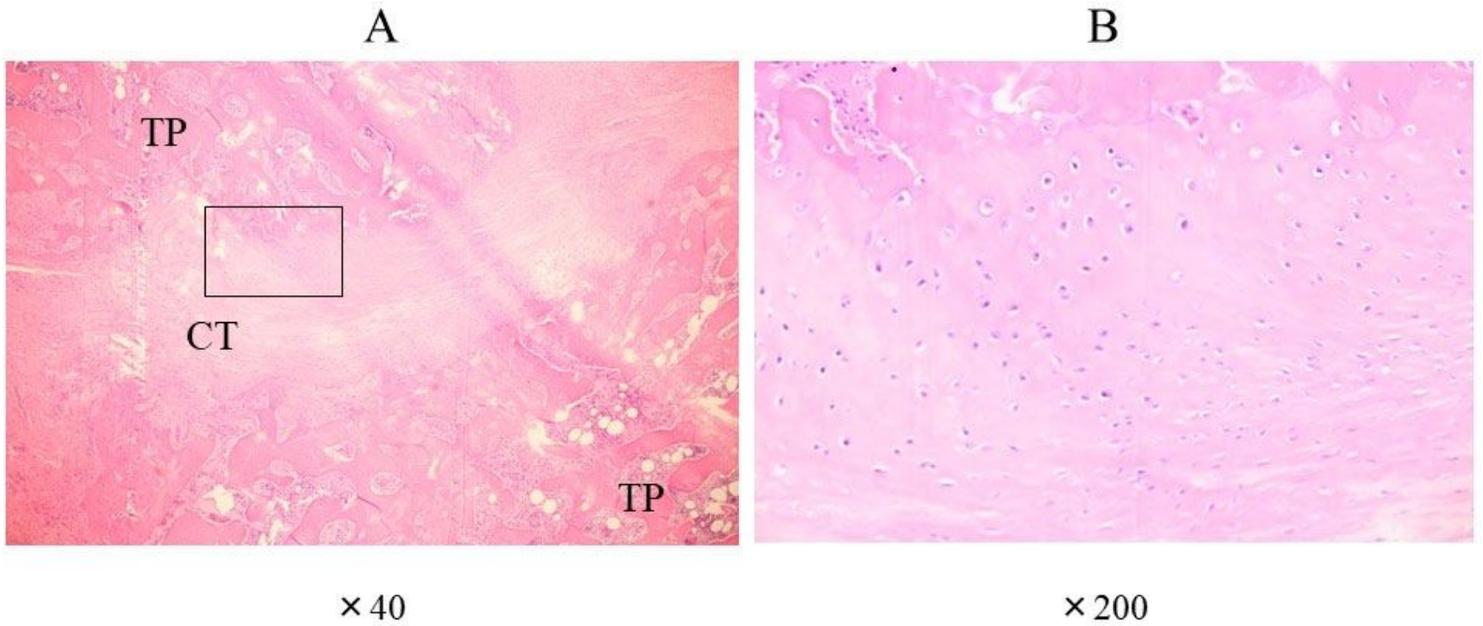
Figure 4.



**Figure 4**

Sagittal histologic cross-section of the L4–L5 transverse processes of rat spines obtained at 8 weeks after posterolateral lumbar fusion surgery. (A) Group I animals, euthanized at 8 weeks postoperatively; magnification 40×. (B) Group I animals, euthanized at 8 weeks postoperatively; magnification 200×. These images clearly demonstrate muscle between the transverse processes for both specimens. Occasional evidence of new bone formation was observed, originating either from the decorticated transverse process or normal remodeling. TP, transverse process; MT, muscle tissue.

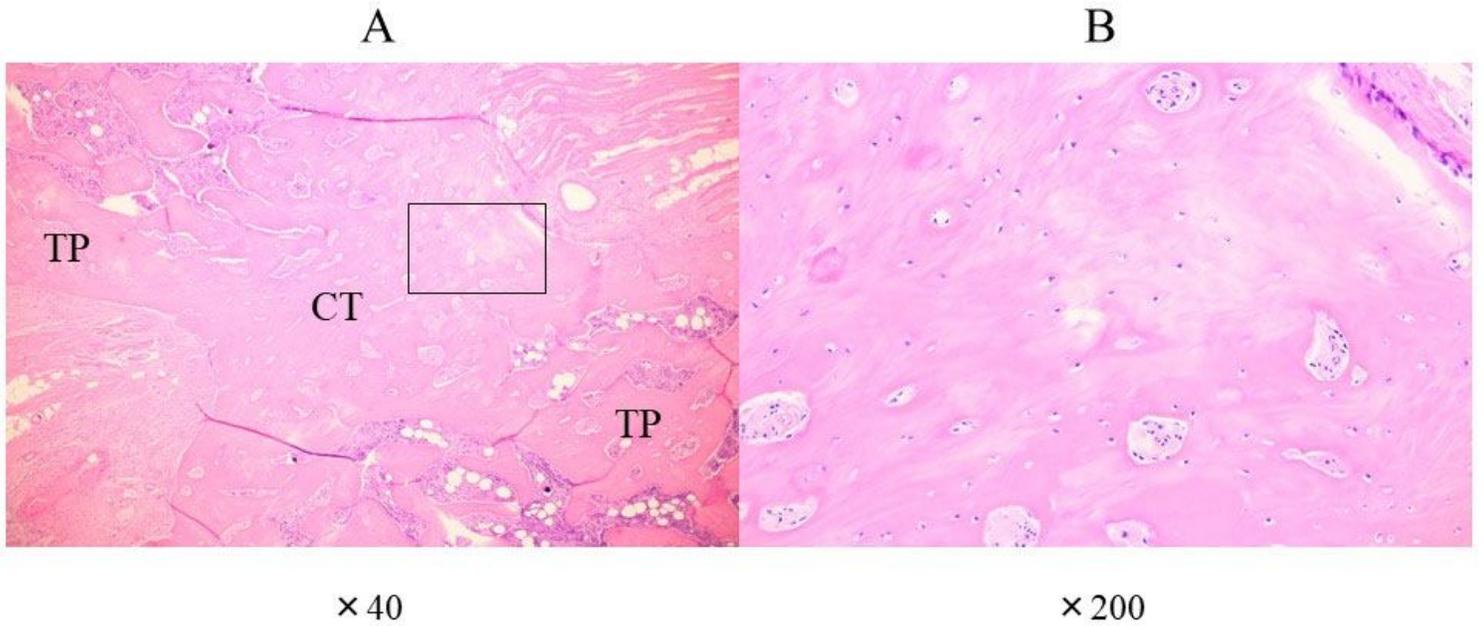
Figure 5.



**Figure 5**

(A) Group II animals, euthanized at 8 weeks postoperatively; magnification 40 $\times$ . (B) Group II animals were euthanized at 8 weeks postoperatively; magnification 200 $\times$ . Distribution of cartilaginous tissue is observed; however, there was fibrosis tissue and muscle fiber between the transverse processes and no evidence of bone fusion. CT, cartilaginous tissue.

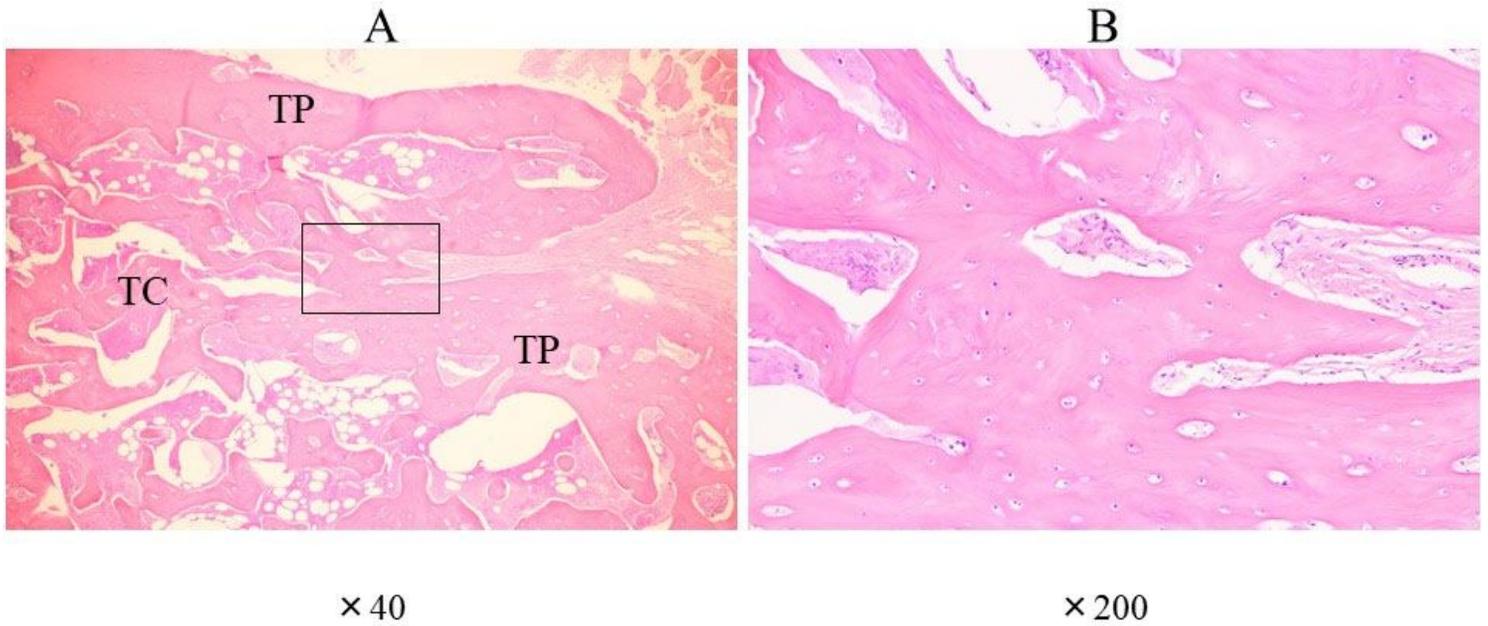
Figure 6.



**Figure 6**

(A) Group III animals, euthanized at 8 weeks postoperatively; magnification 40 $\times$ . (B) Group III animals were euthanized at 8 weeks postoperatively; magnification 200 $\times$ . Analysis of Group III showed distribution of cartilaginous tissue and immature bone formation, but no woven bone between the transverse processes.

Figure 7.



**Figure 7**

(A) Group IV animals, euthanized at 8 weeks postoperatively; magnification 40x. (B) Group IV animals, euthanized at 8 weeks postoperatively; magnification 200x. Group IV showed new bone formation bridging the transverse processes, demonstrating mature osteoid tissue and contracting trabeculae.