INTRODUCTION
Autogenous iliac crest is considered the gold standard for graft material in lumbar fusion. However, pseudoarthrosis may occur in 3 to 25% of cases without instrumentation. Studies show that bone morphogenetic proteins (BMPs) play a critical role in new bone formation and bone remodeling. The activity of endogenous BMPs is regulated by BMP antagonists1. The balance between BMPs and their antagonists determines osteogenic differentiation of progenitor cells. Even through recombinant BMPs have been used as bone graft substitute in spinal fusion, there is no study on BMP antagonists during fusion. Determining the interaction of BMPs and their antagonists may help us understand the intrinsic healing process and design new strategies to facilitate fusion. Additionally, in the study of gene expression during spinal fusion, most work has been focused on the graft itself. The interaction between bone graft and the local micro-environment has been long neglected. Our objectives are to examine the spatiotemporal expression pattern of BMPs and BMP antagonists in a rabbit posterolateral fusion model, to provide information on the reciprocal interaction between the graft and surrounding tissue during bone formation and remodeling.

MATERIAL AND METHODS
Animal model: A single level posterolateral intertransverse process fusion was performed bilaterally at L5-L6 in New Zealand White rabbits, using autogenous iliac crest bone graft2. In the control group, iliac crest, transverse process, and paraspinal muscle were harvested from rabbits without surgery. The rabbits were sacrificed at 1, 2, 4, and 6 weeks postoperatively. Radiography: X-rays were taken on the explanted spines and evaluated by three blinded observers. Histology and Immunohistochemistry (IHC): The fusion masses of the left side were harvested en bloc, fixed, decalcified, and embedded in paraffin. The 4 μm thick tissue sections were processed for H&E, toluidine blue, and immunohistochemical staining. Serial tissue sections were incubated with primary antibodies, including anti-BMP-2, BMP-4, BMP-7, noggin, and chordin antibodies. The sections were then incubated with biotinylated secondary antibody and developed with DAB kit. Real-time Polymerase Chain Reaction (RT-PCR): For the fusion mass of the right side, biopsies were taken from bone graft between the transverse processes (Inner Zone), from bone graft over the transverse process (Outer Zone), from muscle attached to bone graft (Muscle), and from the decorticated transverse process (Transverse Process). Total RNA was extracted from snap-frozen biopsy sample. After RNA was reverse transcribed into cDNA, amplification of BMP-2, BMP-4, BMP-7, noggin, chordin, Sox9, Runx2, and GAPDH was performed. The expression level of each target gene was normalized to the endogenous control GAPDH and expressed as fold change relative to the sample in control group (2^ΔΔCt method). The gene expression levels in the inner zone and outer zone was expressed relative to those of iliac crest in the control group. RT-PCR data were compared by t test with a significant level of P < 0.05.

RESULTS
At 6 weeks, 4 of 6 animals showed radiographic and histologic evidence of fusion. Histology: Three temporal phases of healing, namely inflammatory, reparative, and remodeling, were observed in the spinal fusion process. In the early phase (1 and 2 weeks), the graft site was overwhelmed with hemorrhage, necrotic myofibers, tissue debris, and extensive inflammatory cell infiltration. New bone formation was observed at the muscle-graft interface and adjacent to the decorticated transverse process. In the middle phase (4 weeks), endochondral osteogenesis was predominated, with a number of cartilaginous foci within the inner zone. During the late phase (6 weeks), two transverse processes were continuous with the fusion mass, which showed increased marrow volume and trabecular bone. IHC: During the fusion process, muscle and bone marrow stained positive for all BMPs and their antagonists. Osteocytes in the decorticated transverse process were positive for BMP-2 and BMP-7, weakly positive for chordin, and negative for BMP-4 and noggin. In contrast even at the earliest time point, osteocytes in the transplanted bone graft stained negative for all BMPs and antagonists. In newly formed bone, rimming osteoblasts stained positive for all BMPs and antagonists. However, osteocytes showed positive staining for BMP-2, -7 and chordin, but were negative for BMP-4 and noggin (Fig.1). During endochondral ossification, the peripheral chondrocytes stained positive for all BMPs and antagonists. When differentiated into prehypertrophic chondrocytes, they stained positive for BMP-7 and chordin. The central hypertrophic chondrocytes were negative for all BMPs and antagonists. Fibroblast-like cells, especially those surrounding blood vessels, stained positive for all BMPs and chordin, and weakly positive for noggin. RT-PCR: During the fusion process, muscle Runx2 expression level significantly increased, especially during the early phase. All BMPs and antagonists expression also increased in muscle. The inner zone and outer zone showed significantly lower level of all BMPs in the early phase. However, the level increased significantly at 4 weeks in the outer zone and at 6 weeks in the inner zone. This demonstrates a spatial and temporal difference of BMP gene expression between these two zones. In both zones, noggin level was lower than control, whereas chordin level increased during the fusion process. In the transverse process, the expression level of BMP-2, BMP-4, chordin, and Sox9 were continuously higher than the control, while BMP-7 and noggin level decreased at 2 weeks (Fig.2).

DISCUSSION
This is the first report demonstrating the unique interplay between BMPs and BMP antagonists in a developing spine fusion using a rabbit model. Our results indicate that the muscle surrounding bone grafts is actively involved in osteogenesis3. There is a spatial "lag" effect noted between the inner and outer zone, in terms of histological changes and BMP gene expression. The outer zone demonstrated earlier bone maturation and gene expression than inner zone. This may be attributed to the robust osteoinductive influence of the decorticated transverse process on the adjacent graft4. BMP-positive cells are also noted at the blood vessels. This implies the important role of vasculature and angiogenesis in bone signaling. In addition, the co-localization and reciprocal interaction of BMP and their antagonists suggest a well-controlled system of gene modulation. The differential expression patterns of noggin and chordin indicate that these two antagonists may regulate BMP activity through different mechanisms5.

REFERENCES
5. Gazzerro E et al. Rev Endocr Metab Disord 2006 7:51-65