INTRODUCTION:
Platelet-rich plasma (PRP) is a concentration of platelets and includes several kinds of osteoinductive growth factor such as transforming growth factor-β1 or platelet-derived growth factor. A gelatin β-tricalcium phosphate (β-TCP) sponge was prepared by chemically cross-linking gelatin with β-TCP so that it can release specific growth factors continuously, and simultaneously serve as an osteoconductive scaffold (1). In the meeting last year, we reported that the combination of PRP and the gelatin β-TCP sponge showed a marked osteogenic capacity at 10 weeks postoperatively in a posterolateral fusion model. However, the process of bone fusion using this method is still unclear. Verifying the fusion process employing this method is indispensable in order to use it in a clinical setting. The objective of this study was to assess changes in bone fusion over time employing this method compared to autogenous bone grafting.

METHODS:
Preparation of gelatin β-TCP sponge incorporating PRP
All experimental procedures were approved by the Experimental Animal Center Committee at the authors’ institution. PRP was prepared by twice performing centrifugation of whole blood obtained from Sprague-Dawley (SD) rats. Two hundred μl of PRP was applied to the cube-shaped gelatin-β-TCP sponge, which was then stored overnight at 4℃.

Posterolateral spinal fusion model
A total of 72 SD male rats (aged 8 or 9 weeks) were recruited. The transverse processes (TPs) of L4 and L5 were decorticated using a high-speed burr, and the gelatin β-TCP sponge incorporating PRP (PRP sponge group, n=36) and autologous iliac bone (autograft group, n=36) were implanted bilaterally between TPs.

Radiographic assessment
X-rays and microCT were performed at 2, 4, 6, 8 and 10 weeks after surgery (n=3, respectively), and the bone volume between L4 and L5 TPs was measured at 2, 6, and 10 weeks (n=10, respectively). Individual differences were corrected by dividing the bone volume between the L4 and L5 TPs with that between L1 and L2 TPs. The calculated values were treated as the bone volume ratio.

Biomechanical testing using non-destructive 3-point bending test
The intervertebral mechanical strength between L4 and L5 was assessed using a non-destructive 3-point bending testing at 2, 6, and 10 weeks (n=10, respectively).

Histological analysis
The sagittal tissue sections were processed and stained with HE and safranin O staining at 2, 4, 6, 8, and 10 weeks (n=3, respectively).

Statistical analysis
Data were analyzed employing one-way analysis of variance (ANOVA) with Tukey-Kramer post-hoc testing. A significance level of p<0.01 was used for all analyses.

RESULTS:
Assessment of bone formation by X-ray
In both groups, new bone formation was observed at 4 weeks postoperatively. Subsequently, the shadow density of bone-forming parts intensified over time. Bone fusion was confirmed at 10 weeks after surgery.

Evaluation of bone fusion processes by microCT
In the PRP sponge group, TPs were replaced with new trabecular bone at 4 weeks. At 6 weeks, the new trabecular bone increased and the gap between TPs narrowed. The new trabecular bone further enlarged and TPs fused completely at 10 weeks (Fig. 1). In the autograft group, TPs were replaced with trabecular bone at 4 weeks. At 6 weeks, the bone cortex was generated on the dorsal side. At 8 weeks, the gap between TPs narrowed, and this culminated fusion. There were no significant differences in the bone volume ratio between both groups at 2, 6, and 10 weeks.

Biomechanical strength between vertebrae at fused level
The mechanical strength in the PRP sponge group was the same as that in the autograft group at 2, 6, and 10 weeks.

REFERENCES: