
INTRODUCTION: Platelet-rich plasma (PRP) contains many osteogenic growth factors such as transforming growth factor-β, platelet derived growth factor, or insulin-like growth factor. However, the osteogenic potential of PRP still remains controversial. Gelatin-β-Tricalcium Phosphate (β-TCP) sponges have a pore structure (180-200μm) for bone ingrowth of host bone tissue. Gelatin-β-TCP sponges incorporating bone marrow cells greatly enhanced osteoinductive activity by the controlled release of BMP-2 in a bone defect model (1). The objective of this study is to evaluate the efficacy of gelatin-β-TCP sponges incorporating PRP on bone fusion using a rat posterolateral lumbar fusion model.

METHODS:

Preparation of Gelatin-β-TCP Sponges Incorporating PRP
PRP was prepared by twice performing the centrifugation of 5.0-mL fresh blood obtained from each rat. The supernatant after the second centrifugation was collected as platelet-poor plasma (PPP). Two hundred μL of PRP or PPP was applied to the cube-shaped gelatin-β-TCP sponges, which were then stored overnight at 4°C.

Posterolateral Spinal Fusion Model
All experimental procedures were approved by the Experimental Animal Center Committee at the authors’ institution. The transverse processes (TPs) of L4 and 5 were decorticated using a high-speed burr until they bled from the bone marrow. Gelatin-β-TCP sponges incorporating PRP were implanted bilaterally between TPs of L4–5. A total of 50 Sprague-Dawley rats (age 8 or 9 weeks) were divided into 5 groups (10 rats, 20 specimens per group). Group I: gelatin-β-TCP sponges incorporating PRP. Group II: gelatin-β-TCP sponges incorporating PPP. Group III: gelatin hydrogel incorporating PRP. Group IV: autologous iliac chip bone. Group V: without any implanted biomaterial.

Radiographic Analysis
The fusion between TPs of L4–5 was evaluated using radiography at 5 and 10 weeks after surgery. The amount of bone formation between TPs was quantified by means of a previously developed scoring system [0 indicates minimal or no evidence of new bone formation; 1, immature bone formation, with fusion questionable; and 2, solid-appearing bone, with fusion likely] (2). The animals were sacrificed and the spines were harvested 10 weeks after surgery. Microcomputed tomography (μCT) was performed to evaluate the bone union and the amount of new bone formation at the posterolateral sections between TPs of L4–5.

Manual Palpation and Biomechanical Testing
Manual palpation was done to assess the motion between TPs of L4–5. The absence of motion on both sides was considered to be bone fusion. The solidity of the spine at the fused level was evaluated by a nondestructive 3-point bending test using a testing machine. The bending load at 1 mm deflection was determined from the load-deflection curves.

Histological Evaluation
Sagittal sections of the areas between TPs of L4–5 were evaluated histologically with hematoxylin and eosin staining.

Statistical Analysis
Data were analyzed by the 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:
Radiographic Assessment of Bone Formation
Radiographs showed apparent new bone formation in groups I and IV at 5 weeks and no bone formation in groups II, III, and V. Groups I and IV showed solid-appearing bone at 10 weeks, with fusion likely. In contrast, groups II, III, and V showed no bone formation (Fig. 1). The radiographic scores in groups I and IV were significantly higher than those of the rats in groups II, III, and V. μCT of the posterolateral sections in group I and IV exhibited bony fusion in 18 and 17 sections, respectively. None of the posterolateral sections were fused in groups II, III, and V (Fig. 2). The bone volume of groups I and IV were significantly higher than those of groups II, III, and V.

DISCUSSION:
This study demonstrated that gelatin-β-TCP sponges incorporating PRP could induce solid bone union between TPs. Gelatin can ionically immobilize PRP growth factors and release them in a sustained manner as the microsphere. β-TCP is degradable scaffold that has been clinically tested in bone regeneration therapies because of its inherent osteoconductivity. Gelatin-β-TCP sponges were prepared by chemically crosslinking gelatin with 50 w% β-TCP so that it would have potential to gradually release some growth factors and promote differentiation and proliferation of osteoblasts. In this study, gelatin-β-TCP sponges incorporating PRP showed osteoinductive activity similar to that of autologous iliac bone. These results demonstrate that gelatin-β-TCP sponges incorporating PRP therefore have the potential to be an alternative to autologous bone transplantation for bone fusion.

REFERENCES:

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