

# Bone regeneration on the osteoporotic vertebral fracture model of the rat using Platelet-Rich Plasma and cotton-like $\beta$ -Tricalcium Phosphate

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**INTRODUCTION:** Vertebroplasty, a procedure usually employed for osteoporotic vertebral fractures, has demonstrated utility. However, it is noteworthy that this intervention is accompanied by potential consequences, including neighboring vertebral fractures, material leakage, and adhesion failure, which are contingent upon the specific biomaterial employed. There is a demand for a therapy that is both effective and has a reduced incidence of problems. In a previous study, we experimented using a rat model of osteoporosis-induced lumbar vertebral body bone defects. Specifically, we utilized gelatin  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) sponges in combination with platelet-rich plasma (PRP). This finding revealed successful regeneration of the bone defects. Cotton-like  $\beta$ -TCP has manipulability. Its capacity to stimulate the proliferation of both bone tissue and capillary networks of blood vessels has been observed. However, the impact of cotton-like  $\beta$ -TCP on osteoporotic bone remains uncertain. This study aimed to assess the use of cotton-like  $\beta$ -TCP and PRP in the regeneration of osteoporotic vertebral defects.

**METHODS:** A total of 72 female Sprague-Dawley rats, aged 12 weeks, were utilized in this study to induce osteoporosis by ovariectomy. A 4-mm diameter bone defect was created via drilling into the L3 vertebral body, 8 weeks following OVX therapy. The use of a suitable substance remedied the defect. The participants were categorized into three groups (n=24 each) based on the substance utilized: a control group without any material, a group treated with  $\beta$ -TCP impregnated with PBS (referred to as the PBS group), and a group treated with  $\beta$ -TCP impregnated with PRP (referred to as the PRP group). The third lumbar vertebra was extracted from each rat at four different time points following the surgical procedure, specifically at 0, 4, 8, and 12 weeks. These specimens were subsequently processed for histological examination using Hematoxylin and eosin (HE) staining, tartrate-resistant acid phosphatase (TRAP) staining, and immunostaining techniques. The positive regions of immunostaining were semiquantitatively evaluated and compared in PBS and PRP groups.

**RESULTS:** The histological analysis using HE staining revealed the presence of fibrous tissue in the defect site starting from 4 weeks after the surgical procedure in the control group. By the 8-week mark, vascular tissue had developed within the fibrous tissue. However, at the 12-week time point, the bone repair in the defect site was found to be less extensive compared to earlier stages. In the group treated with PBS, a significant infiltration of multinucleated-like cells was observed in both the bone defect and the periprosthetic area starting from 4 weeks. At 8 weeks, an increase in the cytoplasm of these multinucleated-like cells appeared. By the 12-week mark, the cytoplasm of these cells had further enlarged. In the PRP group, akin to the PBS group, there was observed infiltration of multinucleated cells into the periprosthetic region starting from week 4. Furthermore, there was an increase in the cytoplasm of these cells at 8 and 12 weeks. TRAP staining did not reveal any positively multinucleated-like cells around the artificial bone in the PBS and PRP groups.

The evaluation of osteocalcin (OC) immunohistochemistry was conducted on bone defects. In both the PBS group and the PRP group, the staining of not only the trabecular bone but also the cytoplasm of multinucleated cells surrounding the artificial bone was seen. A semi-quantitative assessment was conducted to evaluate the staining coverage between the PBS and PRP groups. No significant disparity in the osteocalcin area was detected between the two groups at 4 and 8 weeks. However, after 12 weeks, the PRP group exhibited a broader area.

The evaluation of immunohistochemical staining of runt-related transcription factor 2 (RUNX2) in bone defects was conducted. The nucleus of periosteal cells in the defect was stained in the control group. The PRP group had comparable nuclear staining patterns in periosteal and multinucleate-like cells, identical to the PBS group. In the analysis of staining coverage between the groups administered with PBS and PRP, it was observed that the PRP group exhibited significant staining only in the 8th week.

**DISCUSSION:** After four weeks postoperatively, cells were shown to infiltrate into the artificial bone in the vertebral defects of osteoporosis-induced rats in both the PRP and PBS groups compared with the control group. Additionally, fibrous tissue and cells were found to infiltrate the bone defects. Hence, we have deemed the  $\beta$ -TCP material with cotton-like properties to be a valuable scaffold in the osteoporotic bone. The multinucleated cells in the vicinity of the synthetic bone exhibited a lack of TRAP staining but displayed strong immunoreactivity for OC, a protein synthesized by osteoblasts, as well as RUNX2, a protein known to promote osteoblast development. These findings indicate that these cells likely represent an aggregation of osteoblasts. When compared to the PBS group at the 12-week point, PRP was observed to encourage bone growth in osteoporotic vertebrae to evaluate the OC-positive regions. Additionally, PRP was found to promote osteoblast differentiation at an early stage to evaluate the RUNX2-positive regions. This observation suggests that the administration of PRP resulted in the formation of a larger number of osteoporotic vertebrae. The utilization of combined therapy including PRP and cotton-like  $\beta$ -TCP has promise as a viable strategy for addressing osteoporotic vertebral fractures.

**SIGNIFICANCE/CLINICAL RELEVANCE:** (1-2 sentences): Combining PRP and cotton-like  $\beta$ -TCP therapy appears to be a promising approach for treating osteoporotic vertebral fractures.