

## The Effect of Platelet Rich Plasma on the Chondrocyte Apoptosis after Mechanical Injury

**Joon Kyu Lee, MD, PhD, KOREA**

Sahnghoon Lee, MD, PhD, KOREA

Tae Woo Kim, MD, KOREA

Se Hyuk Im, MD, KOREA

Sang Jae Park, MD, KOREA

Sang Cheol Seong, MD, PhD, KOREA

Myung Chul Lee, MD, PhD, KOREA

Seoul National University Hospital

Seoul, KOREA

### Summary:

Platelet rich plasma has the protective effect on the prevention of chondrocyte apoptosis after mechanical injury in ex vivo setting.

### Abstract:

Introduction:

It has been suggested that aberrant chondrocyte apoptosis induced by mechanical injury may play an important role in the pathogenesis of osteoarthritis by affecting the extent of a lesion and repair capability of articular cartilage after injury. Several agents including dexamethasone and IGF-1 have been confirmed to have an effect on the chondrocyte apoptosis prevention through various pathways and preventing the chondrocyte apoptosis may be the important step in the treatment of osteoarthritis. Although numerous theories have been proposed for mechanism of the chondrocyte apoptosis, it is not fully understood. The use of platelet rich plasma (PRP) in clinical orthopedic field regarding articular cartilage injury is getting more and more attention but the mechanism of its action and subsequent results are still not clear. The purpose of this study was to observe whether PRP had the preventive effect on the chondrocyte apoptosis after mechanical injury in ex vivo setting. The effect of PRP application timing (pre-injury, post-injury) was also analyzed.

Methods:

In the first set of experiments, in order to determine the amount of load applied to the cartilage explant in experiments evaluating the effect of PRP on the chondrocyte apoptosis prevention, thirty fresh swine cartilage explants, 5mm in diameter and 3mm in height, were harvested from medial femoral condyle of three fresh swine joints using a punch. The explants were divided into five groups according to loading condition: control, 6.5kg weight from 20cm height, 9.5kg weight from 20cm height, 6.5kg weight from 90cm height, and 9.5kg weight from 90cm height. The load strain was 50% in all groups. The explants were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution for 48 hours before injury and 96 hours after injury. For apoptotic cell count of each explant, the explant was divided into superficial, middle, and deep zones and analyzed with TUNEL stain. Glycosaminoglycan level in culture media of each explant was measured using DMB assay. Scanning electron microscopic images were obtained to assess the morphologies of the cartilage explant. Loading condition of 9.5kg weight from 90cm height was the only condition that resulted in significantly more apoptotic cell count and higher DMB assay result than control. This loading condition was used for the experiments in evaluating the effect of PRP on the prevention of chondrocyte apoptosis. The PRP experiment had eight groups divided by addition of 5% PRP pre injury or post injury and the percentage of FBS added to basal media, and each group was also divided into control and injury subgroups. Four cartilage explants for each group, two for each subgroup, were harvested from pair of fresh swine knee joints. Total five sets of experiments were performed. Culture periods and evaluation methods were same as the first set of experiments.

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### Results:

Chondrocyte apoptosis and GAG levels in media were increased in injury subgroups compared to control subgroups in all groups. Scanning electron microscopy of cartilage explant showed increased surface roughness. Chondrocyte apoptosis rates were highest in the superficial zone of the explant. The number of apoptotic cells and glycosaminoglycan level in media were decreased in groups cultured with additional 5% PRP. The PRP effect on the apoptosis decrease was greater in groups where PRP was added post-injury compared to groups where PRP was added pre-injury and in groups where the basal media contained 1% FBS compared to groups that basal media contained 10% FBS.

### Discussion and Conclusion:

This study demonstrated that PRP had the preventive effect on the chondrocyte apoptosis after mechanical injury. The effect was more distinct when the PRP was used after injury compared to the PRP usage before injury. Although the exact mechanism of PRP action on the prevention of chondrocyte apoptosis is still not clear, various growth factors in PRP might have roles in apoptosis prevention. For instance, IGF-1, one of those growth factors, is a major survival factor and is known to have protective effect on cells from apoptosis under different conditions.