# EFFECT OF PLATELET-RICH PLASMA ON IMPACT-INDUCED CHONDROCYTE APOPTOSIS

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**ABSTRACT**

Objective: To evaluate if the injection of intra-articular platelet-rich plasma (PRP) can reduce impact-induced chondrocyte apoptosis. Methods: A double-blind experimental study was developed in four knees of two adult rabbits. Each knee was injured after anesthesia. Subsequently, 1ml PRP was injected in the right knees and 1ml of normal saline (NS) in the left knees. The animals were euthanized ten days after the intervention. All cartilage was removed from the 4 knees and prepared for analysis in electron microscopy (EM). Results: Four EM samples were obtained. The PRP-injected knees showed apoptosis rates of 47,62% (50/105) and 48,36% (59/122), respectively. NS-injected knees showed 56.67% (17/30) and 70.40% (88/125) of apoptosis. PRP-injected knees had statistically significant less apoptosis (48.02%) than NS-injected ones, (67.74%, $p<0,001$) and odds ratio of 0.439 (95% CI=0.287-0.673). Conclusion: Immediately post-traumatic intra-articular injection of PRP reduces impact-induced chondrocyte apoptosis in rabbits.

**Keywords:** Apoptosis. Cartilage. Platelets-rich plasma. Knee injuries.

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## INTRODUCTION

Articular cartilage has a low regeneration potential, but this process is not yet fully understood. Although several histological studies demonstrate the occurrence of cell death in response to mechanical injury, it has only recently been established whether cell death occurs as a result of necrosis or apoptosis.[1-4]

Apoptosis has been studied extensively in other tissues and cells. Countless known apoptosis inducers have been identified, including chemical agents, cytokines, viral and bacterial pathogens and thermal lesions.[5-8] Apoptosis has been shown to result from mechanical stress in a variety of cells.[9-11] It is a highly regulated and evolutionary programmed process of cell death that plays an essential role in embryonic development and in physiological cell turnover.

Chondrocyte apoptosis was demonstrated in osteoarthritis and in response to mechanical trauma in three in vitro studies.[12-14] These studies concluded that the start of apoptosis in chondrocytes could be one of the early events through which chondrocytes respond to mechanical stimulus. Moreover, other recent investigations demonstrated that apoptosis blocking by pharmacological agents decreased the cell death rate increasing their survival. Some of these agents are represented by caspasis inhibitors,[15] glucosamine,[16] diacerein[17,18] and OP-1.[19]

Soffer *et al*.[20] demonstrated that platelet-derived dose growth factors (PDGF) trigger biological responses that promote bone regeneration, stimulating cell proliferation and decreasing the differentiation of bone cells. Several studies establish direct or indirect proof that platelet-derived products play a substantial role in tissue regeneration.[21,22] They stimulate the cells to produce thrombin, which generates the fibrinogen to form insoluble fibrin. Fibrin clots stimulate the cells to produce type I collagen, thus maintaining a cycle that accelerates tissue regeneration.[23]

While PDGF can increase the viability of bone cells *in vitro*, it still needs to be demonstrated whether this occurs in vivo and whether it can inhibit in post-traumatic apoptosis in the cartilage. The aim of this study is to evaluate whether the intra-articular (IA) injection of platelet-rich plasma (PRP) reduces cartilage degradation after direct trauma.

All the authors declare that there is no potential conflict of interest referring to this article.

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## MATERIAL AND METHODS

The study was conducted at the clinical investigation laboratory of the Orthopedics and Traumatology Institute of the Faculty of Medicine of Universidade São Paulo (LIM-41/IOT/FMUSP). The experimental model followed the ethical principles of COBEA (Colégio Brasileiro de Experimentação Animal), of the American Veterinary Medical Association (AVMA) and of the Comitê de Cuidados e Uso de Animais (IACUC), for maintenance of the animals, anesthesia protocols, analgesia and euthanasia.

## EXPERIMENTAL OSTEOARTHROSIS

Mature New Zealand white rabbits were used in this study. The two rabbits were anesthetized using an intramuscular injection of ketamine (50 mg/kg) and xylazine (100 mg/kg).
In the supine position, the rabbits were submitted to the contusion test for osteoarthritis of Mazières,[17] reproduced three times in each knee. A contusion at the level of the patella and of the medial femoral condyle was caused by the impact of 1 kg of weight released from a height of 1 meter.
Immediately after the contusions, each rabbit received the injection of 1 ml of human-derived PRP in the right knees (denominated 1 and 3) and the injection of 1 ml of normal saline solution (0.9% NaCl) in the left knees (denominated 2 and 4). After the injections, the animals were authorized to remain active, in cages (60 cm X 60 cm x 40 cm), without any immobilization. The animals were rigorously monitored for complications.

## HISTOLOGY

Ten days after the knee contusions, the animals were euthanized. This exact time was selected to evaluate the early phase of post-traumatic apoptosis.[1] Fragments of fresh cartilage were harvested from the patella and from both femoral condyles. The specimens were fixed in 3% glutaraldehyde and in 0.1 M cacodylate at a PH from 7.3 to 7.35 for 2 to 3 hours. After washing in 0.1 M sodium cacodylate at a pH of 7.3, the specimens were fixed in 1 % osmium tetroxide and in 0.1 M sodium cacodylate (pH 7.3) for 1 hour. The specimens were washed again, dehydrated with ethanol then placed in a 50:50 mixture of ethanol/spurn before final incorporation in spurn. After this they were cut and stained with uranyl acetate and citrate, then ultratine sections (60 nm) and analyzed by TEM (transmission electron microscopy (JEOL JEM-1010, Japan)) with enlargement of 1500x.
The investigation by TEM was used to confirm the presence of apoptotic cells and to allow the detailed documentation of their morphological alterations. Both the technician in charge of preparing the cartilage specimens and the pathologist in charge of counting the cells were blind in relation to the study groups (double-blind methodology). For the histological study, each cartilage specimen was divided into random field of the TEM. Normal and apoptotic chondrocytes were counted in these fields. (Figure 1)

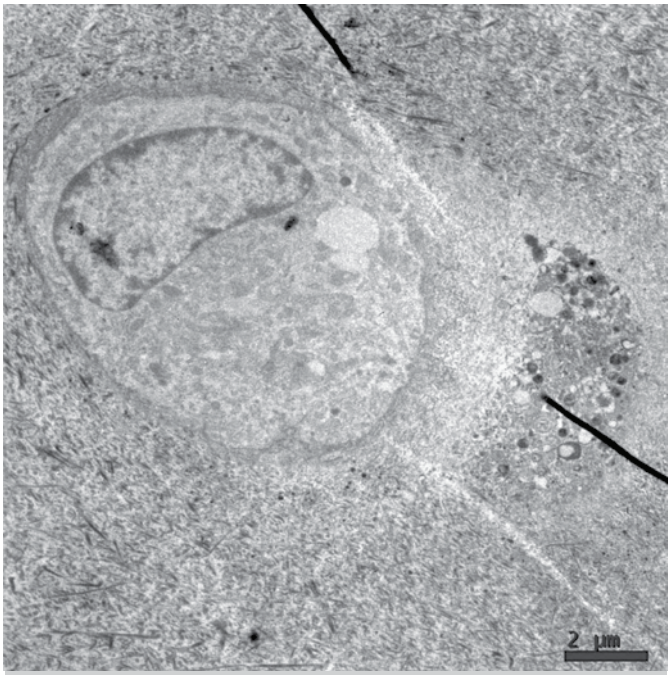


**Figure 1.** *Transmission electron microscopy (TEM) of knee cartilage specimen. White arrow, normal chondrocyte with abundant cytoplasm and normal cellular and nuclear morphology. Black arrow, chondrocyte showing apoptotic alterations including nuclear fragmentation, reduction of cell size and wrinkling of the cytoplasmic membrane.*

## EXTRACTION OF PRP

Apheresis (*Haemonetics*, Braintree, MA, USA) of the peripheral blood of a human volunteer was performed to obtain PRP through the automatic cell separator Haemonetics MCS 9000, specific kit for plateletpheresis 995-E (*Haemonetics* Corp.). The blood was drained to a continuous flow centrifugal separation device. After the blood sedimentation, an optical fiber refractive index analyzer was used to isolate the platelet layer, which was stored in a specific bag. The remaining total blood was reintroduced o in the animal, determining the end of a cycle. The sodium citrate was used as an anticoagulant in a proportion of one for every 9 ml of total blood processed. Generally, two cycles are transported and 70 ml of platelet concentrate are collected.

## STATISTICAL ANALYSIS

The analysis for comparison of the apoptosis proportions between groups was performed by the chi-square test. The sample size estimate resulted in 142 cells for each group.

## RESULTS

Four TEM bases were prepared, with one for each knee. An average 8.75 (7 - 10) meshes were photographed for each grid. Totals of 247 and 158 chondrocytes were counted in the group with introduction of PRP and in the control group, respectively. This number of cells was sufficient to obtain statistical power, as mentioned above.
The knees treated with PRP (knees 1 and 3) revealed the existence of 47.62% (50/105) and 48.36% (59/122) of apoptosis, respectively. On the other hand, in the control group (knees 2

and 4), the apoptosis rates were 56.67% (17/30) and 70.40% (88/125), respectively. (Table 1)

The intra-articular injection of PRP was associated with a significantly lower number of deaths of chondrocytes, 48.02%, in comparison with 67.74% in the control group ($p<0.001$). A protection factor of 56.1 % was demonstrated according to the odds ratio (OR = 0.439; CI95%=0.287-0.673), for the knees treated with PRP. (Figure 2)

**Table 1.** *Number of chondrocytes observed in each knee from the study.*

| Knee | Apoptosis | Normal | Total Cells | % apoptosis |
|---|---|---|---|---|
| 1 | 50 | 55 | 105 | 47.62 |
| 2 | 17 | 13 | 30 | 56.67 |
| 3 | 59 | 63 | 122 | 48.36 |
| 4 | 88 | 37 | 125 | 70.40 |

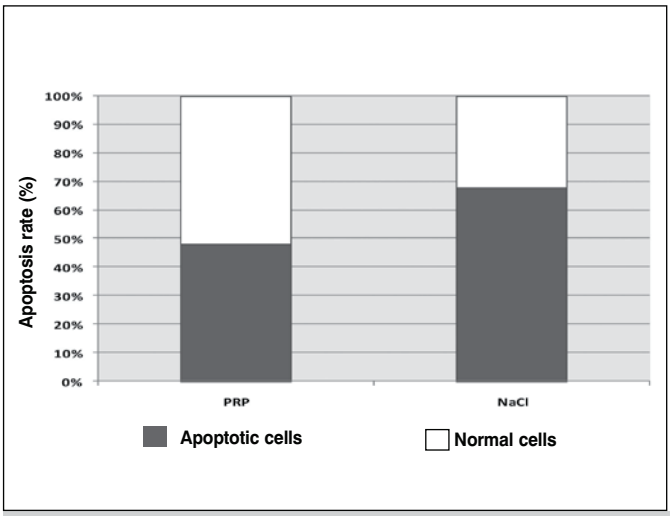


**Figure 2.** *Effect of intra-articular infusion of platelet-rich plasma on chondrocyte apoptosis induced by impact. PRP represents the intervention group and NaCl represents the control group. Significant difference between the two groups ($p<0.001$).*

## DISSCUSION

D'Lima *et al*.[1,24,25] demonstrated the gradual increase of apoptotic cell levels after a traumatic event, offering a potentially therapeutic window. They also demonstrated that apoptosis can be inhibited, suggesting potential for the pharmacological modulation of the effects of cartilaginous lesions, which has already been demonstrated by Mazières *et al*.[17,18] in their contusion model in rabbits. The prophylactic administration (in other words, immediate treatment after the contusion) of diacerein, prevented the destruction of cartilage induced by the impact and presented a chondroprotective effect.

More recently, Chubinskaya *et al*.[19] revealed that the intra-articular injection of OP-1 can prevent post-traumatic apoptosis. Shikhman *et al*.[16] revealed that intra-articular glucosamine has chondroprotective and anti-inflammatory activity in experimental osteoarthrosis.

Platelets were used directly instead of platelet-rich plasma in some studies, while platelet gels and realsates were used in others. The main growth factors stored in []-granules of platelets (platelet-derived growth factors [PDGF]) are the transforming growth factor beta (TGF- ß), the insulin-like growth factor (IGF), the epidermal growth factor (EGF), the fibroblast growth factor (ß-FGF)and the vascular endothelial growth factor (VEGF).[22]

*In vitro* studies revealed that PDGFs are dependent on the number of platelets per ml.[26] O'Neill *et al*.[27] revealed that the method used to harvest the PRP affects the volume of PRP and the concentration of platelets reached. Due to this related efficacy, we used one milliliter of PRP obtained by apheresis. This method (MCS+) has a mean platelet count of $14x10^8$/ml. The results of this study demonstrated that the smaller number needed to show the differences among the samples was 142 cells in each group. In the group injected with PRP there were 247 available cells to count while the control group had 155 cells. (Table 1) Apoptosis was present in 48.02% of the cells after the treatment with PRP and 67.74% of the cells after treatment with normal saline solution (Figure 2), showing a significant statistical difference ($p<0.001$) and at an odds ratio of 0.439 (CI 95 % 0.287-0.673).

D'Lima demonstrated a progressive increase of apoptotic cells after a traumatic lesion.[24] If a single injection of PRP can significantly reduce the number of apoptotic cells, increased dose and frequency should be studied to verify a more expressive chondroprotective action of post-trauma PDGF.

## CONCLUSION

This study does not explain why and how the reduction of apoptosis induced by trauma has occurred, but shows the possibility of using PRP as a protective agent for this type of cartilage lesion.

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