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The effect of platelet-rich plasma on chondrocyte healing in traumatic dislocation of the hip in a rat model

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Ethics Committee Approval
The study was approved by the University of Gaziantep local ethics committee of animal experiments (Approval number: 2019/20), Turkey.
All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Traumatic hip dislocations (THD) are orthopedic emergencies and significant complications such as cartilage degeneration can be reduced with early reduction. This study aimed to examine the effect of platelet-rich plasma (PRP) on cartilage cells in one-, two- or 8-hours prolonged hip dislocation.

Methods: We used 24 Sprague-Dawley rats in this study and divided them into three main groups based on whether their hips were in the protruding position for one, two, or eight hours. Each main group was further divided into two subgroups, with the right hips constituting the experimental group and the left hips, the control group. Traumatic hip dislocation modeling was performed surgically on both hips of the rats under anesthesia in the same session. After both dislocated joints were reduced and the hip capsules were sutured, platelet-rich plasma was administered to the right hips. After 1 week, all rats were sacrificed, their femoral heads were excised and subjected to histopathological examination. Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) testing was used to show chondrocyte apoptosis in the femoral head. The apoptotic index (AI) showing the ratio of cartilage cells to apoptosis was calculated histopathologically. A comparison of apoptotic indices was made between the groups.

Results: The AIs of the one-, two- and eight hours-long hip dislocation groups were 0.012 (0.005), 0.023 (0.011), 0.046 (0.012), respectively ($P<0.001$), while those of the control groups were 0.028 (0.010), 0.077 (0.015), 0.100 (0.016), respectively ($P<0.001$).

Conclusion: In traumatic hip dislocation known to cause chondrocyte apoptosis, PRP provides a significant reduction in the apoptotic index.

Keywords: Apoptosis, Cartilage, Traumatic joint dislocation, Apoptotic index, Platelet-rich plasma (PRP)

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Introduction

Traumatic hip dislocation (THD) leads to pathological sequelae due to both the mechanism of dislocation and the lack of necessary intervention. Conditions such as cartilage damage during trauma to the hip and malnutrition of the femoral head are beyond the control of the surgeon. Other factors, such as the timing and accuracy of the reduction, are positively influenced by the correct evaluation of dislocation as an emergency. Although complications can be reduced with early reduction, and good or excellent results can be seen, long-term complications after hip trauma, such as avascular necrosis (AVN), arthrosis, nerve injury, heterotopic ossification, and re-dislocation [1-4], are common.

PRP can be defined as an autologous platelet concentrate compacted into a small volume of plasma [5, 6]. There are cellular mitogens such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), endothelial cell growth factor (ECGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGFs), and Interleukin-β in the α granules of the thrombocytes. Of these mitogens, IGF-1 is one of the major growth factors associated with cartilage matrix synthesis and cartilage repair. Type 2 collagen regulates the synthesis of proteoglycan and other matrix components [7]. Another growth factor TGF-β1, which is a subgroup of TGF-β, plays a role in bone morphogenic protein-2 (BMP-2) chondrocyte differentiation and matrix maturation. BMP-7, also known as osteogenic protein-1 (OP-1), stimulates type 2 and 4 collagen, aggrecan [ACAN: Cartilage specific proteoglycan core protein (CSPCP) or chondroitin sulfate proteoglycan 1], decorin, fibronectin, hyaluronate, etc., which are cartilage-specific extracellular proteins in chondrocytes [8, 9]. In the light of all these data, we planned our study hypothesizing that externally administered PRP will function as a stimulating factor and reduce chondrocyte apoptosis in chondrocytes with a physiologically low division capacity.

Materials and methods

The animals in this study were obtained from Gaziantep University Experimental Animals Research Center, where all operations except the preparation of specimens were performed.

Twenty-four male Sprague-Dawley rats, 20-24 weeks old and weighing 324-617 grams, were divided into three main groups and randomly assigned to cages in groups of eight, based on whether their hips were in the protruding position for one, two, or eight hours. Each main group was further divided into two subgroups, with the right hips constituting the experimental group and the left hips, the control group. All rats were numbered with ear tags. Both hips of the animals were surgically removed in the same session and reduced after remaining dislocated for the specified periods. Hip capsules were sutured. Thirty µl PRP and twenty µl of phosphate-buffered saline (PBS) were injected into the right capsule after suturing, and only 50 µl of PBS was injected into the left capsule with a Hamilton injector.

Anesthetic technique

After induction anesthesia with 5% isoflurane (Isoflurane-USP, Piramal Critical Care Inc.™, Bethlehem, Pennsylvania, USA), a 1-2% maintenance dose of inhaler anesthesia was administered during the operation with a mask. The respiratory rates and body temperatures of the animals were monitored during surgery. Depth of anesthesia was assessed by the toe pinch test.

PRP preparation technique

Approximately 1 ml of blood was drawn by cannulation from the preoperative right femoral vein of each rat to obtain PRP. The blood samples were placed into a tube containing 2 ml of trisodium citrate to prevent coagulation. PRP was obtained from the collected blood per the 2-stage centrifuge protocol [10].

Surgical technique

A posterior approach was used for access to both hip joints. After incising the skin bilaterally, subcutaneous tissues, and gluteal muscles were divided while protecting both sciatic nerves, and the hip capsule was revealed (Figure 1). Following capsulotomy performed parallel to the coronal plane of the acetabulum and division of *ligamentum teres*, the hip was dislocated posteriorly to prevent spontaneous reduction (Figure 2). Both knees were fixed in extension, and the hips were fixed in internal rotation and adduction to prevent spontaneous reduction. Care was taken to exclude the effects of mechanical trauma on the cartilage during hip dislocation (Figure 3). After hip capsule suturing, 30 µl PRP and 20 µl PBS were injected into the right capsule, and only 50 µl of PBS was injected into the left capsule with a Hamilton injector. The layers were closed properly. After 1 week, both femoral head samples were taken from the old incision line.

Figure 1: Right hip dissection image of the rat a: Marking the skin incision line with a surgical pen, b: Demonstration of the gluteus superficialis muscle, c: Division of the gluteus superficialis muscle, d: The space between piriformis and gluteus medius muscle, e: Demonstration of the sciatic nerve and piriformis after the division of this space

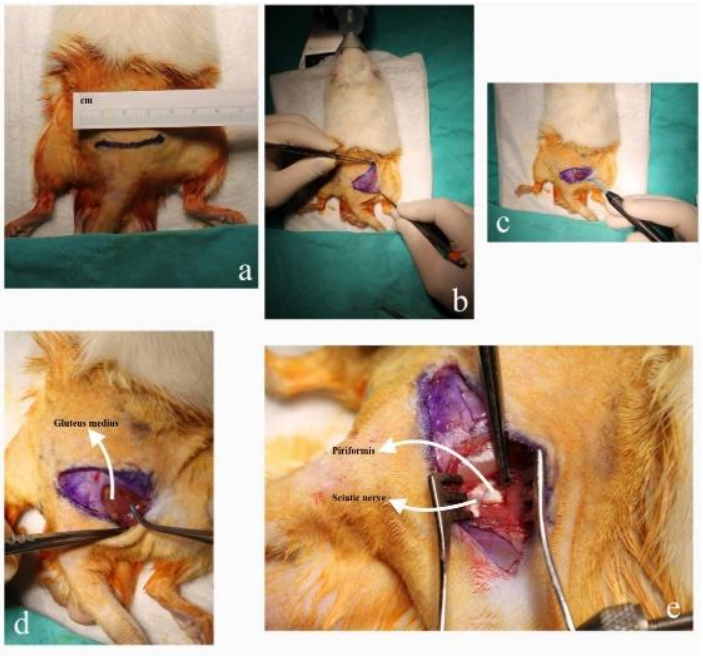


Figure 2: Dissection of rat femoroacetabular capsule. a: Hip capsule after dividing the insertion site of the piriformis muscle from the musculotendinous junction, b: Capsulotomy, c: Distraction of hip joint and demonstration of the femoral head, d: Demonstration of ligamentum teres

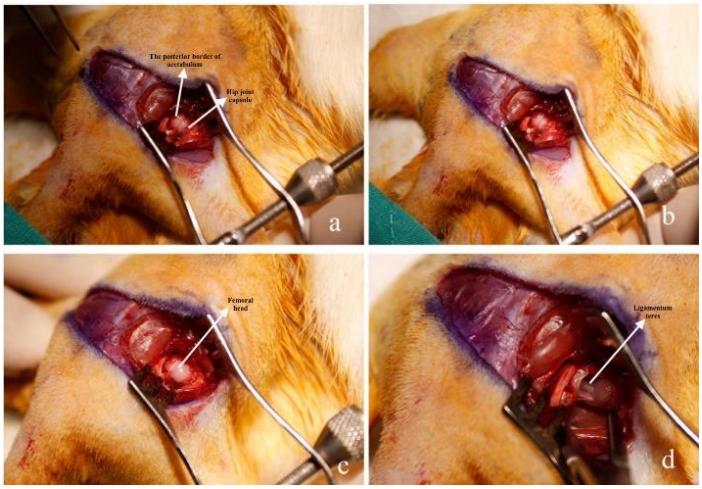
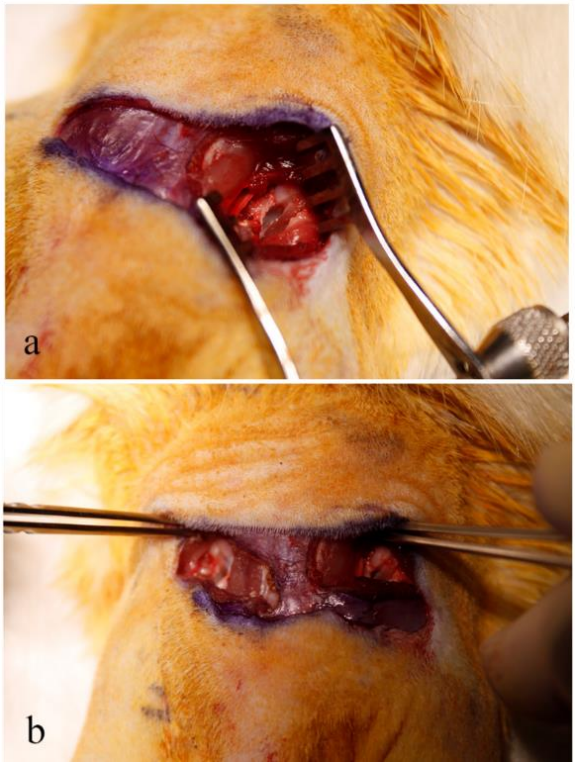


Figure 3: Demonstration of the hip dislocation. a: Right hip dislocated, b: Bilateral hip dislocated



Preoperative and postoperative care

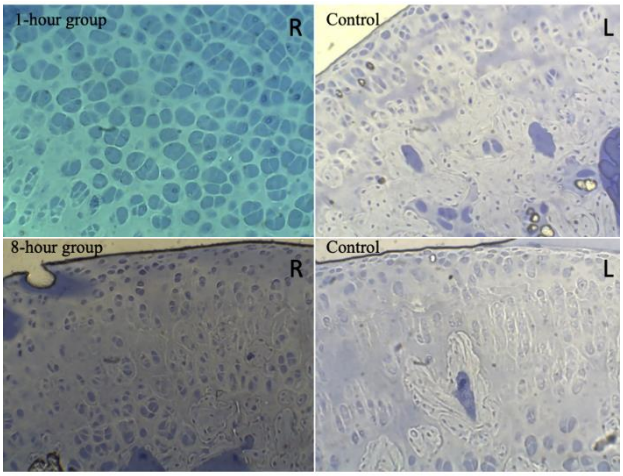
Standard room conditions were provided for all experimental animals, with 12 hours of dark and light cycles. The room temperature was kept between 18-20°C. To prevent hypothermia, the rats were operated under both a light source and a heater, and they were warmed up during recovery. In the postoperative period, the rats were followed up in single cages for 7 days and fed ad libitum.

Histopathological evaluation

The specimens were prepared in Gaziantep University, Department of Pathology.

After both femoral heads of each rat were stored in 10% buffered formaldehyde solution at 4 °C for 7-10 days, the samples were kept in 20% formic acid solution for 2-3 weeks at 4°C for decalcification. The decalcification solution was changed twice a week. The samples were then embedded in paraffin and blocked. The prepared paraffin blocks were sectioned with a 5µm-thickness and stained with hematoxylin-eosin. For the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) test**,** apoptosis was demonstrated with the TUNEL Chromogenic Apoptosis Detection Kit (ABP Biosciences ™, MD, USA, A049). Apoptosis was determined by observing the change in the nuclear chromatin nuclei (Figure 4). When calculating the apoptotic index (AI), the ratio of all chondrocytes to apoptotic cells at ×100 magnifications was considered, and 829-840 cells were counted for each section.

Figure 4: Chondrocytes undergoing apoptosis with TUNEL stain. There is an increased number of apoptotic nuclei in the 1-h and 8-h experimental groups compared with the control groups (All histopathological slides ×100 magnification). TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling



Statistical analysis

The compliance of the data to normal distribution was assessed with the Shapiro Wilk test, and one-way ANOVA-LSD multiple comparison tests were used to compare normally distributed variables in more than two independent groups. The paired t-test was used in the evaluation of normally distributed measurements at two different times. Pearson's correlation coefficient was used to test the relationships between numerical variables.

The minimum required number of animals in each group was five. Power analysis was performed using GPower version 3.1.

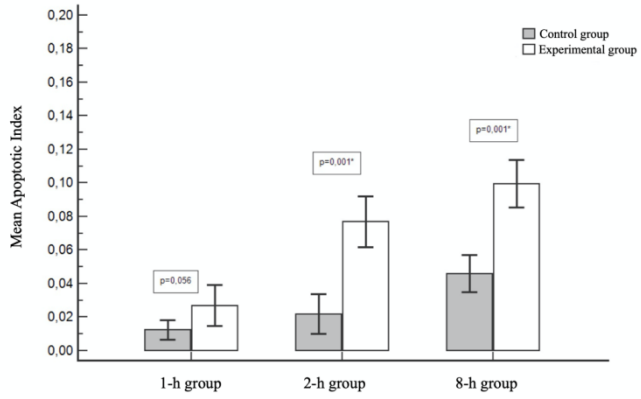
Results

In this study, only the cartilage tissues of the femoral heads, not the acetabular cartilages, were evaluated. The AIs of the one-, two- and eight hours-long hip dislocation groups were 0.012 (0.005), 0.023 (0.011), 0.046 (0.012), respectively (P<0.001), while those of the control groups were 0.028 (0.010), 0.077 (0.015), 0.100 (0.016), respectively (P<0.001) (Figure 5). The AIs of the experimental and control groups were similar in the 1-hour hip dislocation group (P=0.056), while those of the two- and eight-hour hip dislocation groups significantly differed (P<0.001 for both). There was no significant difference between the one- and two-hour hip dislocation experimental groups in terms of AI (P=0.066), but their control subgroups significantly differed (Table 1).

Table 1: Comparison between the experimental and control group in terms of AI and among themselves. AI, apoptotic index

	1-h group	2-h group	8-h group	*P*-value between main groups
Experimental group	0.01 (0.01)	0.02 (0.01)	0.05 (0.01)	0.001
Control group	0.03 (0.01)	0.08 (0.02)	0.1 (0.02)	0.001
P-value between subgroups	0.056	0.001	0.001	

Figure 5: Graph showing femoral head apoptotic indices between the experimental and control groups



Discussion

THD may occur due to cartilage damage caused by mechanical action during dislocation and decreased blood flow in the vessels feeding the subchondral bone [11]. AVN of the femoral head develops because of damage to the vessels feeding the femoral head during dislocation [12]. In addition to all these pathophysiological reasons, the healing process in cartilage limits the classical healing response progressing with bleeding-dependent inflammation and repair due to the avascular structure of the tissue, [13] and progenitor cells in the blood cannot reach the damaged area due to the avascular structure of the cartilage tissue [14]. Apoptosis occurs in the chondrocytes of the femoral head due to the combination of various factors such as the limited number of cells in cartilage tissue and physiologically low division capacity (mitotic activity) of adult chondrocytes [14]. Consequently, the development of osteoarthritis of the hip joint, called coxarthrosis, becomes inevitable.

Chondrocyte apoptosis is clinically important as it occurs in humans and experimentally induced osteoarthritis [14-16] and it has been suggested that chondrocyte death may contribute to the pathogenesis of osteoarthritis [17]. Hashimoto et al. [17] showed that chondrocyte apoptosis increased in the osteoarthritic human joint cartilage, that it may occur even before osteoarthritis becomes evident, and may be related to the severity of cartilage deterioration.

PRP, used in patients with osteoarthritis due to degenerative or secondary reasons, gained increasing popularity in orthopedics [18]. We showed that prolonged dislocation of the joint caused the death of chondrocytes in the form of apoptosis and that PRP given after 1 hour in rats caused a significant decrease in AI and may reduce the long-term complications of concentric reduction.

Although clinical studies are stating that early reduction in hip dislocations should be performed within the first 12-24 hours [19-22], Hougaard et al. [23] and Jasulka et al. [24] stated that it should be performed within the first 6 hours. However, Dreinhofer et al. [3] showed that even if the hip reduction was achieved within the first 6 hours, AVN developed in the femoral head in the long term. In the animal study conducted by Elliot et al. [25], dislocation of the rat hip joint for more than 1 hour caused a significant increase in the apoptotic index. In our study, the apoptotic index significantly increased with the duration of dislocation in both the experimental and the control groups. The mean apoptotic index of the control groups in this study was between 0.04 and 0.12. The femoral head apoptotic index rates in other studies ranged from 0.01 to 0.23 [25-27]. D'Lima thought that this was related to the artifacts in the culture and cutting technique of the pathological specimens [26-28].

Many studies are conducted on PRP and there is no full consensus on this issue among the orthopedic community. Some studies argue that this treatment method does not affect diseases related to orthopedics [18], while others find it effective [29, 30]. In our study, only the experimental group of the 1-hour group did not show a statistically significant decrease compared to the control group. The experimental group of the other groups exhibited a significant reduction in AI ratios compared to the control group.

Limitations

The limitations of our study are the small number of subjects and the lack of a follow-up of long-term traumatic hip dislocation complications. We think that if the financial and ethical problems are overcome, there is a need for further, multicenter studies regarding the effect of platelet-rich plasma on the apoptotic index in humans, with more extensive case series which also evaluate the interobserver and intraobserver variability and reliability.

Conclusion

In the light of all these data, while early reduction is an indispensable treatment method in a patient admitted with THD, the dislocation of the rat hip joint for more than 1 hour significantly increases AI, and PRP application decreases joint degeneration provides a clinically significant improvement by decreasing AI. PRP is a glimmer of hope for the treatment of many diseases in orthopedics and traumatology, but only PRP should not be seen as a definite treatment method.

References

1. Armstrong JR. Traumatic dislocation of the hip joint; review of 101 dislocations. The Journal of bone and joint surgery British volume. 1948;30b(3):430-45.
2. DeLee JC. Fractures and dislocations of the hip. In: Rockwood CA, Bucholz RW, Green DP, Court-Brown CM, Heckman JD, Tornetta P, editors. Rockwood and Green's Fractures in Adults. Philadelphia, PA: Lippincott-Raven; 1996. p. 1756-803.
3. Dreinhofer KE, Schwarzkopf SR, Haas NP, Tscherne H. Isolated traumatic dislocation of the hip. Long-term results in 50 patients. The Journal of bone and joint surgery British volume. 1994;76(1):6-12.
4. Yang RS, Tsuang YH, Hang YS, Liu TK. Traumatic dislocation of the hip. Clinical orthopedics and related research. 1991(265):218-27.
5. Middleton KK, Barro V, Muller B, Terada S, Fu FH. Evaluation of the effects of platelet-rich plasma (PRP) therapy involved in the healing of sports-related soft tissue injuries. The Iowa orthopaedic journal. 2012;32:150-63.
6. Sunitha RV, Munirathnam NE. Platelet-rich fibrin: Evolution of a second-generation platelet concentrate. Indian Journal of Dental Research. 2008;19(1):42-6.
7. Loeser RF. Growth factor regulation of chondrocyte integrins. Differential effects of insulin-like growth factor 1 and transforming growth factor beta on alpha 1 beta 1 integrin expression and chondrocyte adhesion to type VI collagen. Arthritis and rheumatism. 1997;40(2):270-6.
8. Chubinskaya S, Malfait AM, Wimmer MA. Form and function of articular cartilage. In: O'Keefe RJ, editor. Orthopaedic basic science: foundations of clinical practice American Academy of Orthopaedic Surgeons. 4th ed ed. Rosemont, IL2013. p. 183-97.
9. Nishida Y, Knudson CB, Eger W, Kuettner KE, Knudson W. Osteogenic protein 1 stimulates cells-associated matrix assembly by normal human articular chondrocytes: up-regulation of hyaluronan synthase, CD44, and aggrecan. Arthritis and rheumatism. 2000;43(1):206-14.
10. Dhurat R, Sukesh M. Principles and Methods of Preparation of Platelet-Rich Plasma: A Review and Author's Perspective. Journal of cutaneous and aesthetic surgery. 2014;7(4):189-97.
11. Papavasiliou VA. The fate of articular cartilage in traumatic dislocation of the hip. Clinical orthopaedics and related research. 1982(171):287-9.
12. Mehlman CT, Hubbard GW, Crawford AH, Roy DR, Wall EJ. Traumatic hip dislocation in children. Long-term followup of 42 patients. Clinical orthopaedics and related research. 2000(376):68-79.
13. Colwell CW, Jr., D'Lima DD, Hoenecke HR, Fronek J, Pulido P, Morris BA, et al. In vivo changes after mechanical injury. Clinical orthopaedics and related research. 2001(391 Suppl):S116-23.
14. Lotz M, Hashimoto S, Kühn K. Mechanisms of chondrocyte apoptosis. Osteoarthritis Cartilage. 1999;7(4):389-91.
15. Hashimoto S, Takahashi K, Amiel D, Coutts RD, Lotz M. Chondrocyte apoptosis and nitric oxide production during experimentally induced osteoarthritis. Arthritis and rheumatism. 1998;41(7):1266-74.
16. Kourí JB, Aguilera JM, Reyes J, Lozoya KA, González S. Apoptotic chondrocytes from osteoarthrotic human articular cartilage and abnormal calcification of subchondral bone. J Rheumatol. 2000;27(4):1005-19.
17. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis and rheumatism. 1998;41(9):1632-8.

18. Guner S, Buyukbebeci O. Analyzing the effects of platelet gel on knee osteoarthritis in the rat model. Clin Appl Thromb Hemost. 2013;19(5):494-8.
19. Brav EA. Traumatic dislocation of the hip: army experience and results over a twelve-year period. J Bone Joint Surg Am. 1962;44(6):1115-34.
20. Epstein HC. Posterior fracture-dislocations of the hip; long-term follow-up. The Journal of bone and joint surgery American volume. 1974;56(6):1103-27.
21. Pietrafesa CA, Hoffman JR. Traumatic dislocation of the hip. Jama. 1983;249(24):3342-6.
22. Stewart MJ, McCarroll HR, Jr., Mulhollan JS. Fracture-dislocation of the hip. Acta orthopaedica Scandinavica. 1975;46(3):507-25.
23. Hougaard K, Thomsen PB. Traumatic posterior dislocation of the hip--prognostic factors influencing the incidence of avascular necrosis of the femoral head. Arch Orthop Trauma Surg. 1986;106(1):32-5.
24. Jaskulka RA, Fischer G, Fenzl G. Dislocation and fracture-dislocation of the hip. The Journal of bone and joint surgery British volume. 1991;73(3):465-9.
25. Elliott J, Ng de J, Tham SK. Chondrocyte apoptosis in response to dislocation of the hip in the rat model. ANZ journal of surgery. 2006;76(5):398-402.
26. D'Lima DD, Hashimoto S, Chen PC, Colwell CW, Jr., Lotz MK. Impact of mechanical trauma on matrix and cells. Clinical orthopaedics and related research. 2001(391 Suppl):S90-9.
27. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW, Jr. Cartilage injury induces chondrocyte apoptosis. The Journal of bone and joint surgery American volume. 2001;83-A Suppl 2(Pt 1):19-21.
28. D'Lima DD, Hashimoto S, Chen PC, Lotz MK, Colwell CW, Jr. In vitro and in vivo models of cartilage injury. The Journal of bone and joint surgery American volume. 2001;83-A Suppl 2(Pt 1):22-4.
29. Kon E, Mandelbaum B, Buda R, Filardo G, Delcogliano M, Timoncini A, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy. 2011;27(11):1490-501.
30. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. Am J Sports Med. 2010;38(2):255-62.

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