Review Article

Platelet-rich plasma application in chondrogenesis

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Abstract

Platelet-rich plasma (PRP), an autologous derivative of whole blood, has been recently used in surgical treatment. PRP contains growth factors including transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF) and also bioactive proteins that influence the healing of tendon, ligament, muscle, and bone. This article describes the current clinical applications of PRP in chondrogenesis. This study reviews and evaluates the studies that have been published in the field of chondrogenesis. All aspects of using PRP in chondrogenesis are reviewed.

Key Words: Chondrogenesis, growth factors, platelet-rich plasma

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INTRODUCTION

During the last decade, the term platelet-rich plasma (PRP) has received wide and growing attention in the field of regenerative medicine.[1] PRP is defined as the portion of the plasma fraction of blood having a platelet concentration above the baseline value.[2,3] The significance behind using PRP is due to the abundance of growth factors and protein in a well-prepared PRP concentrate involved in tissue engineering.[4] There are some advantages of using PRP. First of all, it is easy to obtain PRP from patient's own blood. Secondly, by regulating the processing technique and activation protocol, it is possible to control the dose of growth factors released on activation.[5] PRP has been used in medical fields such as oral and maxillofacial surgery to enhance hard and soft tissue healing and it has gained attention in orthopedic and sports medicine as a treatment for various problems, including bone, cartilage, ligament, and tendon pathologies.[6-10] Due to the poor regenerative capability of articular cartilage and currently limited clinical treatments, recently cartilage repair, through tissue engineering, has been considered as an alternative approach.

There are two promising cell sources for cartilage tissue engineering: Mesenchymal stem cells (MSCs) and chondrocytes. Both can be differentiated in 3D culture[11,12] in the presence of growth factors such as transforming growth factor-β (TGF-β), insulin-like growth factor-1, and bone morphogenic protein-6 (BMP-6).[13]

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The importance behind using PRP in cartilage tissue engineering field is that PRP is rich in growth factors, including those that promote proliferation of chondrogenic cells and secretion of cartilaginous matrix, such as TGF-β, platelet-derived growth factor (PDGF),[14] insulin-like growth factor (IGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF).[15-17] There are three main types of vesicles detected in a platelet: 1) α-granules, 2) β lysosomes, and 3) dense-core granules. Many of the key ingredients such as growth factors are stored in α-granules.[18] The PRP growth factors and their role in chondrogenesis are given in Table 1.

This review will provide an overview of the studies featuring the role of PRP in chondrogenesis of chondrocytes and stem cells.

PREPARATION OF PRP

In all available PRP techniques, blood is collected with an anticoagulant such as ethylenediaminetetraacetic acid a mixture of citrate, theophylline, adenosine, and dipyridamole (CTAD)[26] and then it is immediately processed by centrifugation. The time for platelet concentrate preparation is about an hour. The first centrifugation step is designed to separate the blood into three layers: Red blood cells (RBCs) are located at the bottom, acellular plasma or platelet-poor plasma (PPP) is at the top, and a "buffy coat" layer appears in between, in which platelets are concentrated. The goal of the subsequent steps is to discard both the RBC layer and the PPP to collect only the buffy coat layer. Finally, the obtained platelet concentrate is applied to the desired site.[27]

Table 1: Growth factors present in platelet-rich plasma and their roles in chondrogenesis

Reference	Growth factors	Function
Grimaud *et al.*,[19] 2002	TGF-β1	Stimulation of proteoglycan and collagen type II synthesis
Fan *et al.*,[20] 2006		Induces chondrogenic differentiation of MSCs
Pufe *et al.*,[21] 2004	VEGF	Cartilage destruction in osteoarthritis
Ferrara *et al.*,[22] 2003		Secreted by hypertrophic chondrocytes
Ataliotis *et al.*,[23] 2000	PDGF	Promotes chondrogenesis at early stages of limb development
Solchaga *et al.*,[24] 2009	IGF-1	Promotes proliferation and chondrogenenic potential of MSCs
Kabiri *et al.*,[25] 2012	FGF-2	Elevates MSCs proliferation and chondrogenic potential

TGF-β1: Transforming growth factor-β1, VEGF: Vascular endothelial growth factor, PDGF: Platelet-derived growth factor, IGF-1: Insulin-like growth factor-1, FGF: Fibroblast growth factor, MSCs: Mesenchymal stem cells

PRP was applied in three forms, including fresh platelets, activated platelets by freeze –thaw, and through thrombin cycles in research and clinical trial. The concentration of the growth factors released was quantified with enzyme-linked immunosorbent assay (ELISA) kits. The highest concentrations of epidermal growth factor (EGF) and fibroblast growth factor (FGF) were found in frozen platelets while the maximum TGF-β1 was detected in thrombin-activated platelets.[28]

EFFECTS OF PRP ON THE CHONDROCYTES' PROLIFERATION AND MATRIX SYNTHESIS

To investigate the effect of PRP on chondrogenesis, it is necessary to consider the changes that occur on the chondrogenic markers such as *SOX9*, aggrecan, and collagen type II. A summary of all the studies is presented in Table 2. It has been shown that using 10% PRP in place of 10% fetal bovine serum in the dulbecco's

Table 2: PRP effects on the chondrocytes *in vitro* and *in vivo*

Reference	PRP preparation	Cell sources	Results
Akeda *et al.*,[29] 2006	10% PRP, activated by thrombin	Porcine chondrocyte (alginate bead)	Increased proteoglycan collagen II promoted proliferation
Satio *et al.*,[30] 2009	3% PRP in gelatin hydrogel microsphere	Rabbit chondrocyte intra-articular (injection)	Suppressed progression of OA in the ACLT rabbit, increased GAG
Zhu *et al.*,[31] 2012	Mice PRP	Rabbit auricular chondrocyte, Cultured to form cell brick	Cell brick-PRP produced more collagen type II and GAG
Spreafico *et al.*,[32] 2009	5% PRP activated by freeze-thaw	Human OA, articular chondrocyte	Promoted the proliferation upregulation of aggrecan, SOX9
Lee *et al.*,[33] 2012	PRP+ hydrogel	Rabbit chondrocyte	Upregulation of SOX9 and aggrecan Anti-inflammatory effect through CB2 and CB1 genes upregulation
Kaps *et al.*,[34] 2002	Platelet supernatant	Bovine articular and nasal chondrocyte	Enhanced proliferation failed to re-differentiation
Choi *et al.*,[35] 1980	Platelet lysate	Rabbit articular chondrocyte	Stimulated cell proliferation, Reduced GAG
Drengk *et al.*,[36] 2009	PRP in different forms	Sheep chondrocyte	Stimulated the proliferation failed to enhance collagen type II
Park *et al.*,[37] 2012	0.1%-20% PRP	Rabbit chondrocyte	Upregulation of SOX9, VEGF, TGF-β ChM-1 in response to 10% PRP
Wu *et al.*,[38] 2007	PRP as a gel	Rabbit chondrocyte	Enhanced GAG production injectable carrier for chondrocyte

OA: Osteoarthritis, ACLT: Anterior cruciate ligament transaction, GAG: Glycosaminoglycan, CB, Cannabinoid receptor, ChM-I: Chondromodullin-I, PRP: Platelet-rich plasma, TGF-β1: Transforming growth factor-β1, VEGF: Vascular endothelial growth factor, SOX9: (sex determining region Y)- box9

Table 3: PRP effects on the MSCs *in vitro* and *in vivo*

Reference	PRP preparation	Cell sources	Results
Mifune *et al.*,[45] 2013	PRP activated by thrombin	Mice MDMSCs	Enhanced production of collagen type II decreased the number of apoptotic cells promoted the proliferation of MDSCs
Kruger *et al.*,[46] 2012	0.1%-100% PRP activated by thrombin	Human CSP	Upregulation of collagen type II aggrecan, link protein, stimulated, extracellular matrix synthesis
Feng *et al.*,[47] 2011	PRP	HUMSCs	Upregulation of collagen type II, Aggrecan
Mishra *et al.*,[16] 2009	10% buffered PRP	HBMSCs	Upregulation of SOX9, aggrecan
Xie *et al.*,[48] 2012	PRP in monolayer PRP as a gel *in vivo*	Rabbit BMSCs and ADSCs	Enhanced the expression of collagen, after 12 weeks in BMSCs group defect filled with cartilage like tissue

MDMSCs: Muscle-derived mesenchymal stem cells, CSP: Cortico-spongious progenitor cell, HUMSCs: Human umbilical cord mesenchymal cell, BMSCs: Bone marrow derived stem cells, ADSCs: Adipose-derived stem cells, PRP: Platelet- rich plasma, SOX9: (sex determining region Y)- box9

modified eagle medium (DMEM) for culturing porcine chondrocytes in alginate beads produced more proteoglycans, glycosaminoglycan (GAG), and DNA.[29] The increased production of GAG was seen when 3% PRP was mixed with gelatin hydrogel and then injected intra-articularly to the rabbit model of osteoarthritis (OA).[30]

Interestingly, PRP in the form of medium supplement *in vitro* and in the gel form that encapsulated cell brick stimulated the synthesis of collagen type II by chondrocytes[29,31] and caused upregulation of the *SOX9* gene expression and when was used in monolayer as a medium supplement or was mixed with hydrogel.[32,33] it has the same effects. The proliferative effect of PRP on chondrocytes from different sources such as bovine articular and nasal septal chondrocytes,[34] rabbit articular chondrocytes,[35] and sheep articular chondrocytes[36] was reported even when PRP was used in the form of platelet lysate. However, the effect of PRP on matrix accumulation was not confirmed in some studies.[34,35] These inconsistent results may be due to the activation form of PRP or the source of chondrocytes. The 10% PRP also stimulates upregulation of TGF-β, VEGF, and chondromodulin-I (ChM-I) by rabbit chondrocytes.[37] This indicates that culturing chondrocytes in the presence of PRP may alter their gene expression profile. PRP can be considered as a successful injectable carrier to study the chondrocytes' differentiation potential. Results showed that PRP as a gel could provide ideal conditions to preserve the chondrocyte phenotype *in vivo* and *in vitro*.[38,39]

THE EFFECTS OF PRP ON THE CHONDROGENESIS AND PROLIFERATION OF MSCs

Due to the problems related to the autologous chondrocyte implantation method[40] for cartilage defect treatment, much attention has been paid to find other cell sources for cartilage tissue engineering. MSCs are a promising cell population for regeneration of mesenchymal tissues such as cartilage. The main chondrogenic inducer MSCs belong to TGF-β family.[41,42] Due to the complexities involved in the safety and efficacy of either exogenous or genetically induced growth factor delivery, investigators are trying to find[43] substitutions such as PRP. The core ingredient of PRP is TGF-β1.[44] A summary of all the studies is presented in Table 3.

Injection of muscle-derived stem cells (MDSCs) with PRP into the knees of rat model of OA produced more collagen type II and decreased the number of apoptotic cells in articular cartilage, promoted proliferation, adhesion, and migration of MDSCs, and finally enhanced the integration of the transplanted cells in the repair process.[45] The effect of human PRP on the chondrogenesis of human subchondral progenitor cells in pellet culture system has been established. At the same time, the upregulation of cartilage hypertrophic marker collagen type X was detectable.[46] It seems that presence of different components of inductive medium such as dexamethasone, insulin–transferrin–selenium (ITS), and ascorbic acid for chondrogenic induction of human umbilical cord derived mesenchymal stem cells (HUCMSCs)[47] and adipose-derived stem cells (ADSCs) (unpublished data) is necessary. The gene expression of aggrecan and *SOX9* were enhanced when bone marrow derived stem cells (BMSCs) were in the presence of 10% buffered PRP[16] This shows that probably activation step is not mandatory. Application of 10% PRP as a medium supplement in monolayer culture of BMSCs and ADSCs caused upregulation of collagen type II, aggrecan, and *SOX9*. When PRP was used as a scaffold for BMSCs and ADSCs, it produced the hyaline cartilage.[48] The mentioned results show the positive effects of PRP in chondrogenesis of MSCs from different tissues.

THE ANTI-INFLAMMATORY EFFECTS OF PRP ON THE CHONDROCYTES

The anti-inflammatory effects of PRP on the chondrocytes seem to correlate with the upregulation of cannabinoid receptor type 1 (CB1). It is reported that CB1 agonists have analgesic and anti-inflammatory effects and reduce joint damage in animal models of arthritis.[33,49,50]

The anti-inflammatory effects of PRP are due to the reduction in the transactivation of nuclear factor-kappa B (NF-κB), the critical regulator of the inflammatory process. Activated PRP has an enhanced concentration of hepatocyte growth factor (HGF) and tumor necrosis factor-α (TNF-α). These growth factors, by disrupting the transactivation of NF-κB, are the key ingredients that contribute to PRP anti-inflammatory effects. The second mechanism is decreasing the expression of inflammatory enzymes cycloxygenase 2 and 4 (COX-2 and COX-4).[51] Decreasing the gene expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) 4 and prostaglandin-endoperoxide synthase (PTGS) 2 is another mechanism used by PRP to avoid inflammation in chondrocytes.[52] It was shown that PRP could have pro-inflammatory effect on the human chondrocytes after the initial reduction of COX-2;[53] may be, PRP has a dual effect on the chondrocytes' response to inflammatory conditions.

CONCLUSION

There are several potential advantages of using PRP in tissue engineering, especially in chondrogenesis. PRP can keep the phenotype and differentiation potential of chondrocytes in terms of proliferation, and synthesis of proteoglycan and collagen type II.

It seems that PRP induce chondrogenesis of MSCs through the secretion of various growth factors, especially TGF-β, since its concentration is high.

The limitation of using PRP is due to two reasons. Firstly, there is not a standard preparation protocol and various platelet activation methods sometimes give different results that do not support each other, thereby making it difficult to compare them. Secondly, there is lack of knowledge about the growth factors and their concentration at which they exert PRP chondrogenic effect. To solve these issues, it is necessary to determine the concentration of PRP growth factors in each study. Since MSCs' chondrogenesis induced by PRP led to production of hypertrophic cartilage, a better understanding of the mechanisms of chondrogenesis is necessary. For OA, however, it needs further investigation.

REFERENCES

1. Arora NS, Ramanayake T, Ren YF, Romanos GE. Platelet-rich plasma: A literature review. Implant Dent 2009;18:303-10.
2. Mehta S, Watson JT. Platelet rich concentrate: Basic science and current clinical applications. J Orthop Trauma 2008;22:432-8.
3. Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent 2001;10:225-8.
4. Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials 2007;28:4551-60.
5. Leitner GC, Gruber R, Neumuller J, Wagner A, Kloimstein P, Hocker P, *et al*. Platelet content and growth factor release in platelet-rich plasma: A comparison of four different systems. Vox Sang 2006;91:135-9.
6. Froum SJ, Wallace SS, Tarnow DP, Cho SC. Effect of platelet-rich plasma on bone growth and osseointegration in human maxillary sinus grafts: Three bilateral case reports. Int J Periodont Restorat Dent 2002;22:45-53.
7. Petrungaro PS. Using platelet-rich plasma to accelerate soft tissue maturation in esthetic periodontal surgery. Compend Contin Educ Dent 2001;22:729-32,34,36.
8. Lopez-Vidriero E, Goulding KA, Simon DA, Sanchez M, Johnson DH. The use of platelet-rich plasma in arthroscopy and sports medicine: Optimizing the healing environment. Arthroscopy 2010;26:269-78.
9. Zhai W, Wang N, Qi Z, Gao Q, Yi L. Platelet-rich plasma reverses the inhibition of tenocytes and osteoblasts in tendon-bone healing. Orthopedics 2012;35:e520-5.
10. Han J, Meng H, Tang J, Li S, Tang Y, Chen Z. The effect of different platelet-rich plasma concentrations on proliferation and differentiation of human periodontal ligament cells *in vitro*. Cell Prolif 2007;40:241-52.
11. Awad HA, Halvorsen YD, Gimble JM, Guilak F. Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells. Tissue Eng 2003;9:1301-12.
12. Ansar MM, Esfandiariy E, Mardani M, Hashemibeni B, Zarkesh-Esfahani SH, Hatef M, *et al*. A comparative study of aggrecan synthesis between natural articular chondrocytes and differentiated chondrocytes from adipose derived stem cells in 3D culture. Adv Biomed Res. 2012;1:24.
13. Sheyn D, Pelled G, Zilberman Y, Talasazan F, Frank JM, Gazit D, *et al*. Nonvirally engineered porcine adipose tissue-derived stem cells: Use in posterior spinal fusion. Stem Cells 2008;26:1056-64.
14. Nikolidakis D, Jansen JA. The biology of platelet-rich plasma and its application in oral surgery: Literature review. Tissue Eng Part B Rev 2008;14:249-58.
15. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. Clin Orthop Relat Res 2011;469:2706-15.
16. Mishra A, Tummala P, King A, Lee B, Kraus M, Tse V, *et al*. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods 2009;15:431-5.
17. Sun Y, Feng Y, Zhang CQ, Chen SB, Cheng XG. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. Int Orthop 2010;34:589-97.
18. Wrotniak M, Bielecki T, Gazdzik TS. Current opinion about using the platelet-rich gel in orthopaedics and trauma surgery. OrtopTraumatol Rehabil 2007;9:227-38.
19. Grimaud E, Heymann D, Redini F. Recent advances in TGF-beta effects on chondrocyte metabolism. Potential therapeutic roles of TGF-beta in cartilage disorders. Cytokine Growth Factor Rev 2002;13:241-57.
20. Fan H, Hu Y, Qin L, Li X, Wu H, Lv R. Porous gelatin-chondroitin-hyaluronate tri-copolymer scaffold containing microspheres loaded with TGF-beta1 induces differentiation of mesenchymal stem cells *in vivo* for enhancing cartilage repair. J Biomed Mater Res Part A 2006;77:785-94.
21. Pufe T, Harde V, Petersen W, Goldring MB, Tillmann B, Mentlein R. Vascular endothelial growth factor (VEGF) induces matrix metalloproteinase expression in immortalized chondrocytes. J Pathol 2004;202:367-74.
22. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669-76.
23. Ataliotis P. Platelet-derived growth factor A modulates limb chondrogenesis both *in vivo* and *in vitro*. Mech Dev 2000;94:13-24.
24. Solchaga LA, Penick K, Goldberg VM, Caplan AI, Welter JF. Fibroblast growth factor-2 enhances proliferation and delays loss of chondrogenic potential in human adult bone-marrow-derived mesenchymal stem cells. Tissue engineering Part A 2010;16:1009-19.
25. Kabiri A, Esfandiari E, Hashemibeni B, Kazemi M, Mardani M, Esmaeili A. Effects of FGF-2 on human adipose tissue derived adult stem cells morphology and chondrogenesis enhancement in transwell culture. Biochem Biophys Res Commun 2012;424:234-8.
26. Yokota M, Tatsumi N, Tsuda I, Nishioka T, Takubo T. CTAD as a universal anticoagulant. J Anal Methods Chem 1900;25:17-20.

27. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma to leucocyte- and platelet-rich fibrin. Trends Biotechol 2009;27:158-67.
28. Burmeister SL, Hartwig D, Limb GA, Kremling C, Hoerauf H, Muller M, *et al*. Effect of various platelet preparations on retinal muller cells. Invest Ophthalmol Vis Sci 2009;50:4881-6.
29. Akeda K, An HS, Okuma M, Attawia M, Miyamoto K, Thonar EJ, *et al*. Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthritis Cartilage 2006;14:1272-80.
30. Saito M, Takahashi KA, Arai Y, Inoue A, Sakao K, Tonomura H, *et al*. Intraarticular administration of platelet-rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee. Clin Exp Rheumatol 2009;27:201-7.
31. Zhu J, Cai B, Ma Q, Chen F, Wu W. Cell bricks-enriched platelet-rich plasma gel for injectable cartilage engineering-an *in vivo* experiment in nude mice. J Tissue Eng Regen Med 2012 [In Press].
32. Spreafico A, Chellini F, Frediani B, Bernardini G, Niccolini S, Serchi T, *et al*. Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. J Cell Biochem 2009;108:1153-65.
33. Lee HR, Park KM, Joung YK, Park KD, Do SH. Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. J Control Release 2012;159:332-7.
34. Kaps C, Loch A, Haisch A, Smolian H, Burmester GR, Haupl T, *et al*. Human platelet supernatant promotes proliferation but not differentiation of articular chondrocytes. Med Biol Eng Comput 2002;40:485-90.
35. Choi YC, Morris GM, Sokoloff L. Effect of platelet lysate on growth and sulfated glycosaminoglycan synthesis in articular chondrocyte cultures. Arthritis Rheum 1980;23:220-4.
36. Drengk A, Zapf A, Stürmer EK, Stürmer KM, Frosch KH. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells. Cells Tissues Organs 2009;189:317-26.
37. Park SI, Lee HR, Kim S, Ahn MW, Do SH. Time-sequential modulation in expression of growth factors from platelet-rich plasma (PRP) on the chondrocyte cultures. Mol Cell Biochem 2012;361:9-17.
38. Wu W, Chen F, Liu Y, Ma Q, Mao T. Autologous injectable tissue-engineered cartilage by using platelet-rich plasma: Experimental study in a rabbit model. J Oral Maxillofac Surg 2007;65:1951-7.
39. Wu W, Zhang J, Dong Q, Liu Y, Mao T, Chen F. Platelet-rich plasma-A promising cell carrier for micro-invasive articular cartilage repair. Med Hypotheses 2009;72:455-7.
40. Dhinsa BS, Adesida AB. Current clinical therapies for cartilage repair, their limitation and the role of stem cells. Curr Stem Cell Res Ther 2012;7:143-8.
41. Mehlhorn AT, Niemeyer P, Kaschte K, Muller L, Finkenzeller G, Hartl D, *et al*. Differential effects of BMP-2 and TGF-beta1 on chondrogenic differentiation of adipose derived stem cells. Cell Prolif 2007;40:809-23.
42. Hashemibeni B, Goharian V, Esfandiari E, Sadeghi F, Fasihi F, Alipur R, *et al*. An animal model study for repair of tracheal defects with autologous stem cells and differentiated chondrocytes from adipose-derived stem cells. J Pediatr Surg 2012;47:1997-2003.
43. Cheng NC, Estes BT, Awad HA, Guilak F. Chondrogenic differentiation of adipose-derived adult stem cells by a porous scaffold derived from native articular cartilage extracellular matrix. Tissue Eng Part A 2009;15:231-41.
44. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. J Craniomaxillofac Surg 2002;30:97-102.
45. Mifune Y, Matsumoto T, Takayama K, Ota S, Li H, Meszaros LB, *et al*. The effect of platelet-rich plasma on the regenerative therapy of muscle derived stem cells for articular cartilage repair. Osteoarthritis Cartilage 2013;21:175-85.
46. Kruger JP, Hondke S, Endres M, Pruss A, Siclari A, Kaps C. Human platelet-rich plasma stimulates migration and chondrogenic differentiation of human subchondral progenitor cells. J Orthop Res 2012;30:845-52.
47. Feng X, Tian S, Sun K, Zhang J, Zhang C, Liu S, *et al*. Effect of platelet lysate on chondrogenic differentiation of human umbilical cord derived mesenchymal stem cells *in vitro*. Zhongguo xiu fu chong jian wai ke za zhi 2011;25:1250-5.
48. Xie X, Wang Y, Zhao C, Guo S, Liu S, Jia W, *et al*. Comparative evaluation of MSCs from bone marrow and adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. Biomaterials 2012;33:7008-18.
49. Lee HR, Park KM, Joung YK, Park KD, Do SH. Platelet-rich plasma loaded in situ-formed hydrogel enhances hyaline cartilage regeneration by CB1 upregulation. J Biomed Mater Res Part A 2012;100:3099-107.
50. Wu CC, Chen WH, Zao B, Lai PL, Lin TC, Lo HY, *et al*. Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokine-inhibited chondrogenesis. Biomaterials 2011;32:5847-54.
51. Bendinelli P, Matteucci E, Dogliotti G, Corsi MM, Banfi G, Maroni P, *et al*. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-kappa B inhibition via HGF. J Cell Physiol 2010;225:757-66.
52. van Buul GM, Koevoet WL, Kops N, Bos PK, Verhaar JA, Weinans H, *et al*. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. Am J Sports Med 2011;39:2362-70.
53. Pereira RC, Scaranari M, Benelli R, Strada P, Reis RL, Cancedda R, *et al*. Dual effect of platelet lysate on human articular cartilage: A maintenance of chondrogenic potential and a transient pro-inflammatory activity followed by an inflammation resolution. Tissue Eng Part A 2013 [In Press].

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