Anti-Inflammatory Effect of Platelet-Rich Plasma on Nucleus Pulposus Cells with Response of TNF-α and IL-1

Ho-Joong Kim,[1,2] Jin S. Yeom,[1,2] Yong-Gon Koh,[3] Jee-Eun Yeo,[3] Kyoung-Tak Kang,[3] Young-Mi Kang,[4] Bong-Soon Chang,[5] Choon-Ki Lee[5]

[1]Department of Orthopaedic Surgery, Seoul National University College of Medicine, 166 Gumiro, Bundang-gu, Sungnam 463-707, Republic of Korea, [2]Spine Center, Seoul National University Bundang Hospital, 166 Gumiro, Bundang-gu, Sungnam 463-707, Republic of Korea, [3]Joint & Spine Laboratory Center, Yonsei Sarang Hospital, 478-3, Bang-bae, Seocho, Seoul 137-820, Republic of Korea, [4]BK21 Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea, [5]Department of Orthopaedic Surgery, Seoul National University College of Medicine, Seoul National University Hospital, 101 Daehangno, Jongno-gu, Seoul 110-744, Republic of Korea

Received 25 August 2013; accepted 9 November 2013
Published online 11 December 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.22532

ABSTRACT: The purpose of this study was to investigate the anti-inflammatory effect of platelet-rich plasma (PRP) with collagen matrix on human nucleus pulposus (NP) cell in response to pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1). NP cells from human disks were cultured in a monolayer and maintained in the collagen matrix prior to the addition of recombinant human IL-1 and TNF-α. After applying IL-1 and TNF-α, PRP prepared by using a commercially available platelet concentration system was added. The response was investigated using real-time PCR for mRNA expression of type II collagen, aggrecan, matrix metalloproteinase-3 (MMP-3), and cyclooxygenase-2 (COX-2). The combination of IL-1β and TNF-α led to decrease of matrix synthesis gene expression such as collagen type II and aggrecan and increase of the degradation gene expression of COX-2 and MMP-3, compared to the control. Consecutive PRP exposure significantly recovered the down-regulated gene expression of collagen type II and aggrecan and significantly reduced the increased MMP-3 and COX-2 gene expression, compared to that of control groups with pro-inflammatory cytokines. The administration of PRP with collagen matrix markedly suppressed cytokine-induced pro-inflammatory degrading enzymes and mediators in the NP cell. It also rescued gene expression concerning matrix synthesis, thereby stabilizing NP cell differentiation. © 2013 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 32:551–556, 2014.

Keywords: platelet-rich plasma; nucleus pulposus cell; interleukin-1; tumor necrosis factor-α

Degenerative changes in human beings are an inevitable phenomenon.[1] Many kinds of musculoskeletal disorders are related to the degeneration of corresponding tissue.[2] Intervertebral disk (IVD) degeneration plays a critical role in almost all lumbar diseases including disk herniation, radiculopathy, spinal stenosis, and low back pain.[3–5] Alterations in the extracellular matrix of the nucleus pulposus (NP) of degenerated IVDs originate from marked decreases in proteoglycan synthesis, increased aggrecan fragment accumulation, decreased type II collagen synthesis, and increased type I collagen synthesis. These factors represent the major pathogenic characterization of IVD degeneration.[5–8]

Furthermore, a variety of inflammatory mediators have been implicated in IVD degeneration, including nitric oxide (NO), interleukin-1 (IL-1), matrix metalloproteinases (MMP), prostaglandin E_2 (PGE_2), tumor necrosis factor alpha (TNF-α), and a group of cytokines.[9,10] The cytokines IL-1and TNF-α are overexpressed in degenerated IVD, which has led to both being implicated in the matrix degradation that characterizes disk degeneration.[11–13] There is some direct evidence that both IL-1 and TNF-α might be involved in matrix degradation.[10,14,15]

Platelet-rich plasma (PRP) is extracted from whole blood and contains mixed growth factors such as tumor growth factor-β (TGF-β), vascular epithelial growth factor, platelet derived growth factor (PDGF), and insulin-like growth factor (IGF), which has been used for soft tissue and bone repair in several clinical settings.[16–19] Concerning IVD, a previous study have demonstrated the potential of PRP on human IVD cells, including promoting cell proliferation, and the redifferentiation and reconstitution of human NP tissue.[20] Moreover, recent reports showed that PRP modulated by collagen matrix has anti-inflammatory potential to rescue the chondrocyte degeneration induced by pro-inflammatory cytokines such as IL-1 and TNF-α.[21,22] Therefore, given that the IL-1 and TNF-α also have a critical role in the degeneration of IVD as pro-inflammatory cytokines, we hypothesized that PRP might react with pro-inflammatory cytokine to maintain the homeostasis of NP cell. The purpose of this study was to investigate the anti-inflammatory effects of PRP with collagen matrix on human NP cells responded to pro-inflammatory cytokines.

MATERIALS AND METHODS

All procedures were approved by institutional review board at the hospital.

Nucleus Pulposus Cell Isolation and Culture

After a diagnosis of degenerative disk disease, such as lumbar spinal stenosis or spondylolisthesis, disk tissues were harvested from three patients (age 52–62 years, mean age; 58.1 years) at the time of surgery. The disks were classified as grade 3 disk degeneration according to the Thompson scale.[23] The NP was dissected, washed with Hank's balanced salt solution (HBSS), and transported to the laboratory within 30 min. Specimens from individual patients were

Conflicts of interest: None.

Correspondence to: Jin S. Yeom (T: +82-31-787-7195; F: +83-31-787-4056; E-mail: highcervical@gmail.com)

minced into small fragments. Cells (hNP) were then isolated and expanded in monolayer with collagen matrix. The cells were cultured in F-12/D-MEM containing 1% fetal bovine serum (FBS), 1% penicillin–streptomycin (PS), and 25 μg/ml L-ascorbic acid under standard conditions (37°C, 5% CO_2, 95% air, bicarbonate buffering to maintain pH 7.2). Cell culture materials were purchased from Introgen/Gibco (Seoul, Korea) unless noted otherwise, and hNP cells were used at passage 1 or 2. Experiments began after the cells had been in monolayer culture for at least 1 week, when collagen II mRNA expression had been restored, and after culture in serum free medium with insulin–transferrin–selenium for 24 h.

PRP Preparation

Blood samples from three healthy donors were collected after participants gave informed consent. PRP was prepared by means of a GPS III System (Biomet, Inc., Warsaw, IN) according to the manufacturer's protocol. Upon addition of 22.8 mM $CaCl_2$ 1:10 (v/v), clotting caused activated platelets to release growth factors. Supernatant was collected, which was assigned to PRP releasate. The baseline platelet and white blood cell concentrations of whole blood and PRP releasate were measured on a clinical ADVIA2120 (SIMENS, Ireland, UK). PRP releasate was subsequently stored in aliquots of 1.5 ml at −80°C for further experiments and growth factor analyses. Baseline PDGF-AB was measured using the PDGF-AB Quantikine ELISA Kit (R&D Systems, Minneapolis, MN), while TGF-β1 was measured using human TGF-β1 Immunoassay Quantikine (R&D Systems, Abingdon, UK). Two sample dilutions were measured for growth factor analyses.

PRP-Conditional Medium Preparation and Responses to Cytokines

NP cells were seeded into 24-well plates at a density of 2×10^4 cells/ml and treated with or without PRP, while the experimental controls were cultured in F-12/DMEM supplemented with 1% FBS. *As positive control, cells were treated with PRP without pro-inflammatory cytokines*. Cells were then pre-treated with IL-1β (1.0 ng/ml) and TNF-α (2.0 ng/ml) for 48 h *which represented degenerative disk environment as negative control*, followed by PRP releasate (5%, 10%) for 48 h. The optimum concentrations of IL-1β, TNF-α, and PRP releasate were selected based on results obtained from cytotoxicity studies using a broad concentration range for each reagent.[21,24,25]

Gene Expression

Gene expression was analyzed by real-time RT-PCR using an ABI PRISM® 7900HT, Sequence Detection System (Applied Biosystems, Foster City, CA). The reactions were performed with primers and tested to ensure specificity with melt curve analysis performed with each assay (Table 1). Type II collagen and aggrecan were analyzed to assess matrix gene expressions, while the cyclooxygenase-2 (COX-2) and MMP-3 genes were analyzed to assess inflammatory reactions and matrix degradation, respectively. Real-time RT-PCR reactions were performed in duplicate in 96-well plates in a volume of 25 μl using the reagents and optimized protocol of the VeriQuest SYBR Green qPCR Master Mix (Affymetris, Santa Clara, CA). Cycle threshold (C_t) values were obtained, and the data were normalized to GAPDH expression using the $\Delta\Delta C_t$ method to calculate relative mRNA levels for each gene.

Statistical Analysis

All values were reported as the mean ± standard deviation of the analysis results of separate cultures. Experiments were performed for three PRP donors on each NP cell donor. Statistical analysis was performed using a mixed-model analysis of variance. The treatment was considered as a fixed factor, and donor was considered as a random factor. The post hoc analysis was also used. The effects of IL-1β/TNF-α, and/or PRP were calculated in separate models. A p value <0.05 was considered statistically significant. All statistical analyses were performed by using the SPSS 20.0.0 statistics package (SPSS, Inc., Chicago, IL).

RESULTS

White Blood–Cell Concentration and Baseline Analysis of Growth Factors in PRP

The mean white blood cell and platelet concentration in the whole blood samples were $5.3 \pm 1.4 \times 10^3/\mu l$ and $198.4 \pm 54.2 \times 10^3/\mu l$, respectively. On average, PRP had a platelet concentration 3.9 times higher ($773.8 \pm 156.43 \times 10^3/\mu l$) than that of whole blood and contained more white blood cells ($15.81 \pm 6.3 \times 10^3/\mu l$; Table 2). The growth factors PDGF-AB and TGF-1β were abundantly present in PRP releasate (Table 2).

Effects of Pro-Inflammatory Cytokines on NP Cell Gene Expression

The combination of IL-1β (1.0 ng/ml) and TNF-α (2.0 ng/ml) led to decrease of matrix synthesis gene expression, such as collagen type II and aggrecan, more than the control. Contrarily, these cytokines increased the degradation gene expression of COX-2 and MMP-3 compared to the control (Fig. 1). Among these changes of gene expressions, however, COX-2 gene expression only demonstrated a statistically significant increase compared to the control (Fig. 1).

Table 1. Primers Used for RT-PCR Analysis of Gene Expression

Gene	5′ Forward 3′	5′ Reverse 3′
GAPDH	ACCCACTCCTCCACCTTTGAC	TCCACCACCCTGTTGCTGTAG
Aggrecan	AAGAATCAAGTGGAGCCGTGTGTC	TGAGACCTTGTCCTGATAGGCACT
Type II collagen	ATGACAATCTGGCTCCCAAC	GAACCTGCTATTGCCCTC
MMP-3	CAAGGAGGCAGGCAAGACAGC	GCCACGCACAGCAACAGTAGG
COX-2	TCCACCAACTTACAATGCTGACTATG	AATCATCAGGCACAGGAGGAAGG

Table 2. Analyses of White Blood Cell Concentration and Growth Factors in PRP Releasate

	Concentration $\times 10^3/\mu l$	
Blood Components	PLT	WBC
Blood component analyses		
Whole blood	198.4 ± 54.2	5.3 ± 1.4
PRPr	773.8 ± 156.43	15.81 ± 6.3
	PDGF-AB/BB	TGF-β1
PRP releasate analyses (pg/ml)		
Growth factors (mean ± SD)	$15{,}623.4 \pm 4{,}584.3$	$54{,}028.7 \pm 6{,}520.7$

Values are mean ± SD. SD, standard deviation.

Changes in other gene expressions after the administration of pro-inflammatory cytokines were not statistically significant.

Effects of PRP on NP Cell Gene Expression

Exposing the control group to PRP caused only statistically nonsignificant increases in matrix gene expression of type II collagen and aggrecan compared to the values of the control group without pro-inflammatory cytokines (Fig. 1). Expression of the inflammatory COX-2 gene decreased after PRP administration, but not in a statistically significant manner. However, PRP significantly decreased MMP-3 gene expression from the control without pro-inflammatory cytokines (Fig. 1).

Retrieving Effect of PRP Against Pro-Inflammatory Cytokines on NP Cell Gene Expression

PRP exposure significantly recovered the down-regulated gene expression of collagen type II and aggrecan, compared to control groups with pro-inflammatory cytokines (Fig. 1). Conversely, PRP significantly reduced the increased MMP-3 gene expression, compared to that of control groups with pro-inflammatory cytokines. Furthermore, COX-2 gene expression significantly decreased in the PRP administration, compared to the control group with pro-inflammatory cytokines (Fig. 1).

DISCUSSION

Clinical evidence suggests that PRP could have beneficial therapeutic effects on hard and soft tissue healing, due to growth factors stored in the platelets.[18,26] Moreover, previous studies reported an anti-inflammatory role of activated PRP.[21,26] This work was intended to investigate both the retrieving and regenerative potentials of PRP on NP cells against pro-inflammatory cytokines such as TNF-α and IL-1.

As expected, our data demonstrated that a combination of IL-1β (1.0 ng/ml) and TNF-α (2.0 ng/ml) induced degenerative changes in NP cells. These changes included decreased expression of genes regarding matrix synthesis such as collagen type II and aggrecan and the increase expression of degradation genes such as COX-2 and MMP-3. TNF-α and IL-1 are well known as major pro-inflammatory cytokines. The NP

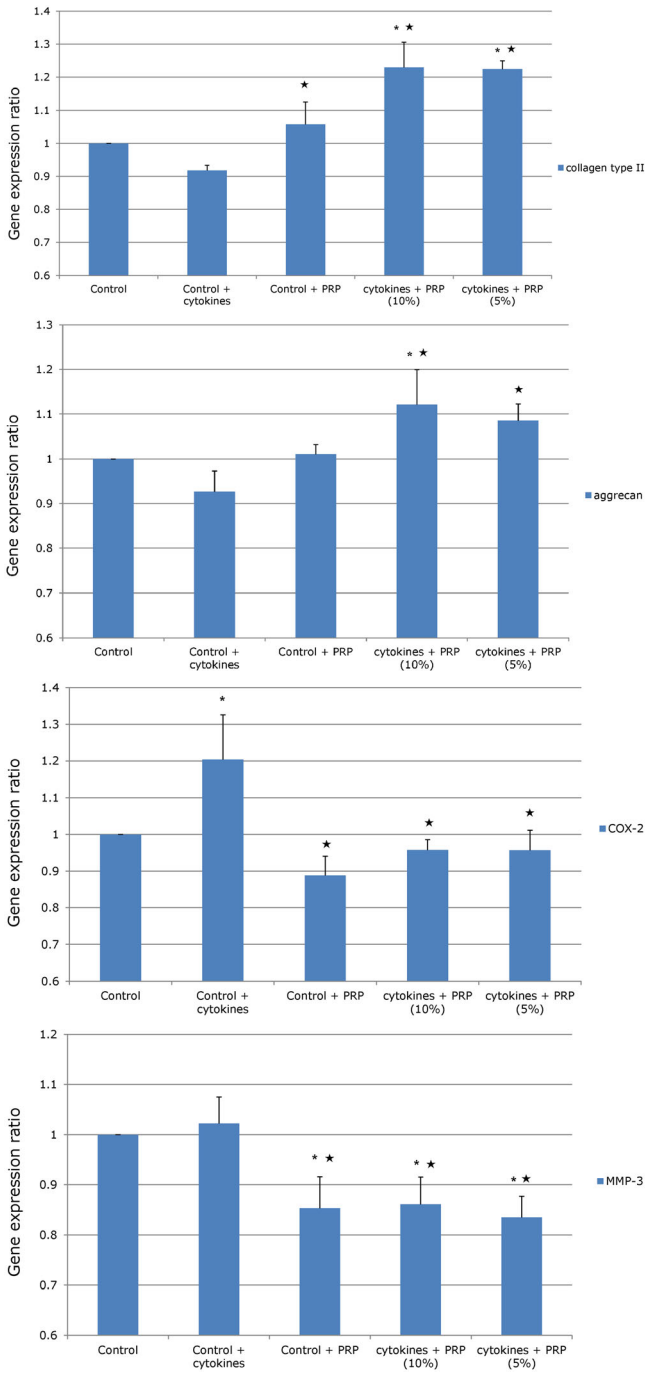

Figure 1. The effect of pro-inflammatory cytokines and/or platelet-rich plasma releasate (PRPr) on gene expression of NP cell. Expressions were normalized to GAPDH mRNA levels (control + cytokine; NP cell treated with IL-1β and TNF-α, control + PRP; NP cell adding to PRPr, cytokines + PRP [5%, 10%]; NP cell treated with IL-1β and TNF-α for 48 h, followed by PRP [5%, 10%] for 48 h, asterisk (*) indicates $p < 0.05$ compared with control, ★ indicates $p < 0.05$ compared with control + cytokines). (a) Collagen type II gene expression, (b) Aggrecan gene expression, (c) COX-2 gene expression, and (d) MMP-3 gene expression.

tissue of herniated IVDs secreted increased levels of nitric oxide, prostaglandin E2, IL-1, IL-6, IL-8, and TNF-α.[9,10,14] Previous studies showed that IL-1 and TNF-α are key cytokines mediating matrix degradation in the IVD.[12,14] Even though this study revealed trends consistent with previous studies, however, there was no statistical significance in collagen type II, aggrecan, and MMP-3 changes, except in COX-2 gene expression. This appears to be because we used human degenerated NP cells from elderly donors. Lower numbers of pro-inflammatory cytokine receptors were found in the aging NP cells than in young and mature adult cells.[27] Another reason for modest increase of MMP-3 gene expression by the addition of pro-inflammatory cytokines can be explained by pre-stimulation of degenerated disk. The NP cell used in the current study was obtained from degenerated disk of grade 3 of Thompson scale, which means that the disk had already been exposed to pro-inflammatory cytokines related to degeneration in vivo. A previous study by Kang et al. is also in line with this result, showing that addition of IL-1 caused only nonsignificant increase of MMP production in the degenerated disk, whereas the addition of IL-1 caused the dramatic increase of MMP production in the nondegenerated disk.[10] For these reasons, human NP cells in our culture model could mask the effect of IL-1β and TNF-α. Collectively, the current results corroborate clinical findings regarding the association between pro-inflammatory cytokines and disk degeneration, and the NP cell after adding pro-inflammatory cytokines in this study also represent the in vitro degenerative state of disks, indicating that the decrease in extracellular matrix gene expression, mediated by TNF-α and/or IL-1.

Previous studies have reported favorable results regarding the regenerative potentials of PRP on NP cells or chondrocytes.[20,28] They described consistent results of increased gene expression of collagen type II and aggrecan upon PRP addition in both disk cells and chondrocytes.[28,29] The key players in these anabolic effects are TGF-β and IGF-1.[20] The present study showed only statistically nonsignificant increases in matrix gene expression after exposure to PRP, compared to the values of control. Given that the NP cell donors were older than 50-year old, and that TGF-β and IGF-1 receptors is deplete during the aging process,[30] the results are not contrary to those of previous studies.[20,28] Furthermore, several reports have insisted that PRP reduces type II collagen mRNA gene expression as a culture additive.[31,32] This effect is explained by the fact that an increase in proliferation negatively influences the differentiation status of a cell.[31] Therefore, the current result is not surprising considering the diametrically opposed reports regarding the effect of PRP on type II collagen and aggrecan mRNA gene expression.[31,32]

As previously mentioned, the degenerative disk state and inflammatory-mimicking environment were created by adding IL-1β and TNF-α to NP cells in culture. In this state, the effect of PRP on NP cells was more pronounced than in the exposure of PRP to the control group without pro-inflammatory cytokines. The PRP exposure did not only significantly restore the down-regulated gene expression of collagen type II and aggrecan caused by pro-inflammatory cytokines, but also significantly reduced the increased COX-2 and MMP-3 gene expression. These results imply that PRP retrieved the pro-inflammatory cytokine-induced suppression of chondrocyte gene expression and ameliorated the increased catabolic and inflammatory activity represented by MMP-3 and COX-2, respectively. Previous similar reports support the anti-inflammatory role of PRP, and have shown that TGF-β, the major growth factor in PRP, could counteract the effects of IL-1, including degradation of type II collagen and proteoglycan produced by chondrocytes.[33] Bendinelli et al.[26] has determined the anti-inflammatory role of PRP due to the presence of hepatocyte growth factor (HGF). These results are consistent with our conclusion that PRP restores a microenvironment that is beneficial for maintaining NP cell homeostasis from IL-1 and TNF-α inhibition. From the literature review, previous studies have focused mainly on the anabolic effect and/or tissue repair of PRP.[16,18,19,28] However, degenerative disk diseases shift toward the catabolic side when patients require treatment.[24] Emphasis should be placed on understanding not only the anabolic PRP effects, but also the preventive effect of PRP on catabolic disk degeneration in the environments related to aging. From this perspective, the current study firstly elaborates on potential applications for restoring the anabolic–catabolic imbalance in degenerative disk disease with aging.

It should be noted that collagen matrix was used as the frame of the major cartilage matrix for NP cell culture in the current study. Previous studies have demonstrated that collagen matrix promoted chondrogenesis in mesenchymal progenitor cells[34] and induced redifferentiation of articular chondrocytes and NP.[20,21,26] Cell–matrix interactions play an important role in maintaining cartilage homeostasis. The extracellular matrix can support prime tissue functions and maintain physical microenvironments of NP cells. According to previous studies,[35,36] cell membrane receptors also play a crucial role in the cell–matrix interaction. Integrin-α, -β, and CD44 showed high affinity to collagen fibril and may facilitate chondrogenesis. In the current study, the protection efficacy of PRP on chondrogenic-specific gene expression was enhanced by the assistance of collagen matrix, which provided a physiological microenvironment beneficial for NP cell survival.

The current study has certain limitations. First, we should have assessed the plausible mechanism of anti-inflammatory effects of PRP. However, several previous studies already suggested that PRP exerts its anti-inflammatory role in the chondrocyte by inhibiting nuclear factor kappa B.[24,26] Second, the present study only included NP cells, not AF cells, in response to PRP. In tissue repair, the anti-inflammatory and

regenerative effects of PRP seem to be as important in AF cells as in NP cells. This is because degenerative annular lesions, such as annular tear, play pivotal roles in the degeneration cascade of disks.[4,15] Furthermore, a previous study reported that the effect of PRP on matrix synthesis was more pronounced in AF cells than in NP cells.[28] Given that the annulus fibrosus matrix is rich in collagen, which was used as an enhancer of the PRP effect, the response of AF cells to PRP under inflammatory or degenerative environments should be delineated in future research. Third, the present study demonstrated only changes in gene expression after exposure to pro-inflammatory cytokines and/or PRP in the in vitro model. Because the increased gene expression does not always results in the increased protein synthesis especially in the elderly,[24] this would be a limitation of the current work. Finally, several recent studies have focused on variability in the cellular composition of PRP, in which leukocytes in PRP increased catabolic signaling molecules.[37,38] Given the concentration-dependent function of white blood cells to be either beneficial or harmful in PRP treatments, the present results should be considered only based on the cellular characteristics of current platelet preparations, in that the current PRP was nonautologous and contained substantial WBC.

In conclusion, the findings of the current study corroborate our hypothesis that PRP can suppress cytokine-induced pro-inflammatory degrading enzymes and mediators. In addition, PRP can rescue gene expression concerning matrix synthesis, thereby stabilizing NP cell differentiation. Although there have been several reports suggesting the anti-inflammatory effect of PRP on chondrocytes, this is the first study regarding the interaction between human NP cells with pro-inflammatory cytokines and PRP. Although additional studies are needed before clinical application, the current results encourage further study of PRP as a conservative, autologous, and feasible treatment for early stage of degenerative disk disease.

REFERENCES

1. Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 90:7915–7922.
2. Loeser RF. 2010. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clin Geriatr Med 26:371–386.
3. Kirkaldy-Willis WH, Wedge JH, Yong HK, et al. 1978. Pathology and pathogenesis of lumbar spondylosis and stenosis. Spine 3:319–328.
4. Farfan HF, Huberdeau RM, Dubow HI. 1972. Lumbar intervertebral disc degeneration: the influence of geometrical features on the pattern of disc degeneration—a post mortem study. J Bone Joint Surg Am 54:492–510.
5. Hangai M, Kaneoka K, Kuno S, et al. 2008. Factors associated with lumbar intervertebral disc degeneration in the elderly. Spine J 8:732–740.
6. Anderson DG, Tannoury C. 2005. Molecular pathogenic factors in symptomatic disc degeneration. Spine J 5:260S–266S.
7. Ahsan R, Tajima N, Chosa E, et al. 2001. Biochemical and morphological changes in herniated human intervertebral disc. J Orthop Sci 6:510–518.
8. Roberts S, Caterson B, Menage J, et al. 2000. Matrix metalloproteinases and aggrecanase: their role in disorders of the human intervertebral disc. Spine 25:3005–3013.
9. Cassinelli EH, Hall RA, Kang JD. 2001. Biochemistry of intervertebral disc degeneration and the potential for gene therapy applications. Spine J 1:205–214.
10. Kang JD, Stefanovic RM, McIntyre LA, et al. 1997. Toward a biochemical understanding of human intervertebral disc degeneration and herniation. Contributions of nitric oxide, interleukins, prostaglandin E2, and matrix metalloproteinases. Spine 22:1065–1073.
11. Le Maitre CL, Freemont AJ, Hoyland JA. 2005. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 7:R732–R745.
12. Hoyland JA, Le Maitre CL, Freemont AJ. 2008. Investigation of the role of IL-1 and TNF in matrix degradation in the intervertebral disc. Rheumatology 47:809–814.
13. Weiler C, Nerlich AG, Bachmeier BE, et al. 2005. Expression and distribution of tumor necrosis factor alpha in human lumbar intervertebral discs: a study in surgical specimen and autopsy controls. Spine 30:44–53.
14. Sguin CA, Pilliar RM, Roughley PJ, et al. 2005. Tumor necrosis factor-alpha modulates matrix production and catabolism in nucleus pulposus tissue. Spine 30:1940–1948.
15. Shinmei M, Kikuchi T, Yamagishi M, et al. 1988. The role of interleukin-1 on proteoglycan metabolism of rabbit annulus fibrosus cells cultured in vitro. Spine 13:1284–1290.
16. Jenis LG, Banco RJ, Kwon B. 2006. A prospective study of Autologous Growth Factors (AGF) in lumbar interbody fusion. Spine J 6:14–20.
17. Mehta S, Watson JT. 2008. Platelet rich concentrate: basic science and current clinical applications. J Orthop Trauma 22:432–438.
18. Alsousou J, Thompson M, Hulley P, et al. 2009. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. J Bone Joint Surg Br 91:987–996.
19. Eppley BL, Woodell JE, Higgins J. 2004. Platelet quantification and growth factor analysis from platelet-rich plasma: implications for wound healing. Plast Reconstr Surg 114: 1502–1508.
20. Chen W, Lo W, Lee J, et al. 2006. Tissue-engineered intervertebral disc and chondrogenesis using human nucleus pulposus regulated through TGF-beta1 in platelet-rich plasma. J Cell Physiol 209:744–754.
21. Wu C, Chen W, Zao B, et al. 2011. Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokine-inhibited chondrogenesis. Biomaterials 32:5847–5854.
22. Woodell May J, Matuska A, Oyster M, et al. 2011. Autologous protein solution inhibits MMP-13 production by IL-1β and TNF-α stimulated human articular chondrocytes. J Orthop Res 29:1320–1326.
23. Thompson J, Pearce R, Schechter M, et al. 1990. Preliminary evaluation of a scheme for grading the gross morphology of the human intervertebral disc. Spine 15:411–415.
24. van Buul GM, Koevoet WLM, Kops N, et al. 2011. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. Am J Sports Med 39:2362–2370.
25. Studer RK, Vo N, Sowa G, et al. 2011. Human nucleus pulposus cells react to IL-6: independent actions and amplification of response to IL-1 and TNF-α. Spine 36:593–599.
26. Bendinelli P, Matteucci E, Dogliotti G, et al. 2010. Molecular basis of anti-inflammatory action of platelet-rich plasma on

human chondrocytes: mechanisms of NF-κB inhibition via HGF. J Cell Physiol 225:757–766.
27. Bachmeier BE, Nerlich AG, Weiler C, et al. 2007. Analysis of tissue distribution of TNF-α, TNF-α-receptors, and the activating TNF-α-converting enzyme suggests activation of the TNF-α system in the aging intervertebral disc. Ann N Y Acad Sci 1096:44–54.
28. Akeda K, An HS, Pichika R, et al. 2006. Platelet-rich plasma (PRP) stimulates the extracellular matrix metabolism of porcine nucleus pulposus and anulus fibrosus cells cultured in alginate beads. Spine 31:959–966.
29. Spreafico A, Chellini F, Frediani B, et al. 2009. Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. J Cell Biochem 108:1153–1165.
30. Matsunaga S, Nagano S, Onishi T, et al. 2003. Age-related changes in expression of transforming growth factor-beta and receptors in cells of intervertebral discs. J Neurosurg 98:63–67.
31. Drengk A, Zapf A, Strmer EK, et al. 2009. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells. Cells Tissues Organs 189:317–326.
32. Gaissmaier C, Fritz J, Krackhardt T, et al. 2005. Effect of human platelet supernatant on proliferation and matrix synthesis of human articular chondrocytes in monolayer and three-dimensional alginate cultures. Biomaterials 26: 1953–1960.
33. Demoor FM, Boittin M, Redini F, et al. 1999. Differential effects of interleukin-1 and transforming growth factor beta on the synthesis of small proteoglycans by rabbit articular chondrocytes cultured in alginate beads as compared to monolayers. Mol Cell Biochem 199:69–80.
34. Chen CW, Tsai YH, Deng WP, et al. 2005. Type I and II collagen regulation of chondrogenic differentiation by mesenchymal progenitor cells. J Orthop Res 23:446–453.
35. Reid DL, Aydelotte MB, Mollenhauer J. 2000. Cell attachment, collagen binding, and receptor analysis on bovine articular chondrocytes. J Orthop Res 18:364–373.
36. Cao L, Lee V, Adams ME, et al. 1999. beta-Integrin-collagen interaction reduces chondrocyte apoptosis. Matrix Biol 18:343–355.
37. Mazzocca AD, McCarthy MBR, Chowaniec DM, et al. 2012. Platelet-rich plasma differs according to preparation method and human variability. J Bone Joint Surg Am 94:308–316.
38. Sundman EA, Cole BJ, Fortier LA. 2011. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med 39:2135–2140.