Systematic Review

Platelet-Rich Plasma in the Pathologic Processes of Cartilage: Review of Basic Science Evidence

Niall A. Smyth, M.D., Christopher D. Murawski, B.S., Lisa A. Fortier, D.V.M., Ph.D., Brian J. Cole, M.D., M.B.A., and John G. Kennedy, M.D., F.R.C.S.

Purpose: The purpose of this study was to systematically review the basic science evidence for the use of platelet-rich plasma (PRP) in the treatment of pathologic processes of cartilage, both as an adjunct to cartilage repair and as a conservative management strategy for osteoarthritis, with the intent of determining the effect of PRP and whether a proof of concept for its use has been established to facilitate further investigation at a clinical level. **Methods:** Using the terms "platelet-rich plasma OR PRP OR autologous conditioned plasma OR ACP AND cartilage OR chondrocytes OR chondrogenesis OR osteoarthritis OR arthritis" we searched EMBASE and PubMed/Medline in April 2012. Two authors performed the search, 3 authors independently assessed the studies for inclusion, and 2 authors extracted the data. Extracted data included cytologic analysis of PRP, study design, and results. **Results:** Twenty-one studies (12 in vitro, 8 in vivo, one in vitro and in vivo) met the inclusion criteria. The effects of PRP in these studies included increasing chondrocyte and mesenchymal stem cell proliferation, proteoglycan deposition, and type II collagen deposition. PRP was also found to increase the cell viability of chondrocytes and the migration and chondrogenic differentiation of mesenchymal stem cells (MSCs) and to inhibit the effect of catabolic cytokines. In vivo, PRP was used as an adjunct to concomitant surgical management, including microfracture surgery and implant, scaffold, and graft insertion. Not all studies concluded that PRP has a positive effect on cartilage repair. **Conclusions:** The current basic science evidence suggests that PRP has several potential effects on cartilage repair and osteoarthritis, and a proof of concept has been established. Well-designed randomized controlled trials (RCTs) are needed to extrapolate this evidence to the clinical setting.

Platelet-rich plasma (PRP) is an autologous blood product produced by the centrifugation of whole blood, thereby yielding a concentration of platelets that is increased to higher than baseline values.[1] The physiologic role of platelets in the natural healing process has led to the investigation of PRP as a treatment for a variety of musculoskeletal indications.[2] In this regard, PRP has been controversial because its widespread use has continued for a myriad of conditions without supporting evidence established by high-quality randomized controlled trials (RCTs). To date, PRP has been used in the orthopaedic setting to treat Achilles tendinopathy, anterior cruciate ligament tears, plantar fasciitis, and epicondylitis, as well as to augment spinal fusion, bone healing, rotator cuff repair, arthroplasty, and cartilage regeneration.[3-12] Of these indications and to our knowledge, only epicondylitis is supported by consistent level I clinical evidence.[13,14]

A recent meta-analysis by Sheth et al.[15] examined the evidence for the use of PRP for orthopaedic indications. The authors concluded that although there was a trend in the literature favoring the use of PRP, an analysis of the available evidence revealed uncertain results because of a lack of standardization of outcome measures, PRP production, and investigative protocols. Understandably, there has been a call for higher level RCTs to establish the clinical efficacy of PRP. However, before embarking on time-consuming and expensive RCTs, there is a necessity to establish a proof of concept for the use of PRP in each potential pathologic condition.

Articular cartilage pathologic processes present orthopaedic surgeons with a difficult clinical challenge. Cartilage provides a smooth gliding surface that uniformly distributes load over the surface of weight-bearing joints, thereby facilitating normal joint motion.[16]

From Hospital for Special Surgery (N.A.S., C.D.M., J.G.K.), New York, New York; College of Veterinary Medicine (L.A.F.), Cornell University, Ithaca, New York; Rush University Medical Center (B.J.C.) Chicago, Illinois, U.S.A.

The authors report that they have no conflicts of interest in the authorship and publication of this article.

Received January 28, 2013; accepted March 5, 2013.

Address correspondence to Niall A. Smyth, M.D., Hospital for Special Surgery, 523 East 72nd St, Ste. 507, New York, NY 10021, U.S.A. E-mail: smythn@hss.edu

0749-8063/1382/$36.00

http://dx.doi.org/10.1016/j.arthro.2013.03.004

Once damaged, it has a poor potential for spontaneous healing because of an inherent avascularity, leading to focal cartilage lesions and osteoarthritis.[17,18] This has led to a multitude of surgical treatment modalities that attempt to heal cartilage lesions through either reparative or replacement techniques.[19,20] The surgical options available, however, have produced suboptimal results, partly because of the difficulty in stimulating cartilage repair.[21-27] This has led to research on the use of biological adjuncts that may improve cartilage repair, including PRP.[28,29] Additionally, PRP has been investigated as an intra-articular injection for the treatment of cartilage lesions and osteoarthritis of weight-bearing joints.[11,30,31] Despite this, there is no high-level clinical evidence to support the use of PRP as an adjunct to cartilage repair or as an intra-articular injection to treat osteoarthritis. The aim of this study was to systematically review the basic science evidence for the investigation of PRP in pathologic processes of cartilage, including both as an adjunct to cartilage repair and as a conservative management strategy for osteoarthritis, with the intent of determining the effect of PRP and whether there is sufficient evidence to establish a proof of concept to proceed with further investigation at a clinical level.

Methods

Literature Search

Using the terms "platelet-rich plasma OR PRP OR autologous conditioned plasma OR ACP AND cartilage OR chondrocyte OR chondrogenesis OR osteoarthritis OR arthritis" we searched the EMBASE (1974 to present) and the PubMed/MEDLINE electronic databases in April 2012. The reference lists of included studies were also reviewed and compared with the collected studies to ensure that no pertinent articles were omitted.

Inclusion Criteria

Studies were included if they fulfilled the following criteria: they (1) studied the effect of PRP or a similar concentrated platelet product defined as a blood product with platelet concentration elevated to higher than baseline, (2) established a control with which to compare PRP, and (3) were published in a peer-reviewed journal. All variations of PRP preparations were included and comprised activated PRP, PRP releasate, PRP gels, and nonactivated PRP liquid. All review articles and clinical studies, including RCTs and case series, were excluded from this review. Two authors performed the literature search, and 3 authors independently reviewed the search results. The title and abstract were reviewed for all search results, and potentially eligible studies received full-text review. All 3 assessing authors agreed on studies meeting criteria for inclusion.

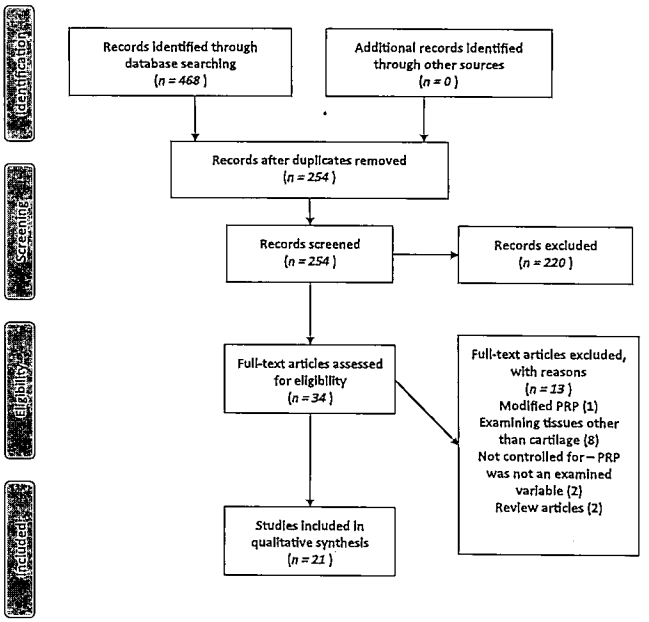

Fig 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram for literature selection.[73]

Data Extraction

A standardized data sheet was developed and 2 authors extracted all pertinent information, including cytologic analysis of PRP, study design, and results.

Results

After the removal of duplicates, the literature search yielded 254 relevant citations. After abstract review, 220 articles were excluded because they failed to meet the inclusion criteria. Of the 34 articles remaining for full-text review, 13 were excluded because they studied tissues and cells not relevant to cartilage repair, did not control for PRP administration, or were review articles (Fig 1). Thus, 21 studies were determined to be eligible for inclusion.[32-52] There were 12 in vitro [34-37,39,41,43,46,48-50,52] and 8 in vivo[32,33,38,40,42,44,47,51] studies. One study included both in vitro and in vivo components.[45] Table 1 describes the in vitro and in vivo studies' design and results.

Nineteen of the 21 studies (90.5%) reported the method by which PRP was prepared. One study reported basic cytologic analysis of PRP, including platelet, white blood cell (WBC), and red blood cell (RBC) counts. One study reported both platelet count and WBC count. Thirteen studies reported platelet count alone. Six studies (28.6%) made no mention as to the composition of the PRP used (Table 2). Multiple variations of PRP preparation were reported, including activated PRP, PRP releasate, PRP gels, and nonactivated PRP liquid.

Table 1. Results of Systematic Review

Study	PRP Cytologic Findings	Study Design	Results
Lee et al.[32] 2012	N/A	Cartilage lesion in rabbits (n = 4). PRP combined with hydrogel and chondrocytes *v* hydrogel and chondrocytes alone, implanted in defect site. Euthanized at 4 wk. PCR for growth factors and cell viability of chondrocytes in culture assessed at 3, 7, and 14 d.	PRP enhanced chondrocyte viability and maturation. PRP upregulates *SOX9*, *CB2*, *CHM1*, and aggrecan.
Milano et al.[33] 2012	Mean whole blood platelet concentration: 428 ± 96 × 10^3/mL Mean PRP platelet concentration: 868 ± 112 × 10^3/mL	Cartilage lesion in sheep (n = 30). Microfracture model in 2 groups. Group 1 → 5 weekly injections of PRP. Group 2 → No further treatment. Euthanized at 3, 6, and 12 mo.	ICRS gross scores significantly improved in PRP group at all time points. Modified O'Driscoll histologic scores significantly greater in PRP group at all time points, except hyaline cartilage. Significantly greater cartilage stiffness in PRP group at all time points.
Sitek et al.[34] 2013	N/A	Human chondrocyte + fibrinogen graft *v* chondrocyte + fibrinogen + PRP graft. Cultured for 3 wk.	No difference in cell viability between groups. Cells viable throughout.
Krüger et al.[35] 2012	Platelet concentration range: 0.6-1.3 × 10^{10}/mL Leukocyte concentration: <0.3 × 10^4/mL	Human subchondral progenitor cells isolated postmortem. PRP-containing serum separated from progenitor cell–containing serum by porous membrane. Incubated for 20 h. Progenitor cells cultured with 5% PRP for 28 d.	PRP stimulated migration and chondrogenic differentiation of human subchondral mesenchymal progenitor cells. PRP increased proteoglycan and type II collagen deposition.
Park et al.[36] 2012	Platelet concentration range: 6-10 × 10^6/μL	Rabbit chondrocytes cultured in PRP (1%, 5%, 10%, and 20%) and assessed at 1, 3, 5, and 10 d.	At 5% concentration, PRP increased chondrocyte proliferation (40%) and cell viability (100% increase), increased aggrecan gene expression, and increased mRNA expression of TGF-β and BMP. PRP increased staining for type II collagen and safranin O.
van Buul et al.[37] 2011	Mean platelet concentration in whole blood: 125 × 10^6/mL Mean platelet concentration in PRP: 845 × 10^6/mL Mean WBC concentration in whole blood: 5.55 × 10^6/mL Mean WBC concentration in PRP: 36.57 × 10^6/mL Mean RBC concentration in whole blood: 4,463 × 10^6/mL Mean RBC concentration in PRP: 610 × 10^6/mL	Human osteoarthritic chondrocytes cultured for 48 h with and without IL-1β, with varying concentrations of PRP (0%, 1%, and 10%).	PRP decreased IL-1β–mediated inhibition of type II collagen and aggrecan gene expression. PRP decreased IL-1β–mediated increase in *ADAMTS* and *PTGS2*. PRP inhibited NF-κβ activation by IL-1β.
Lippross et al.[38] 2011	Platelet concentration: up to 10^6/μL	Inflammatory arthritis model in pigs (n = 15). Ten pigs immunized; 5 pigs nonimmunized controls. Five treatment animals with PRP injections at d 56 and d 70. Animals euthanized on d 84.	Improvement in Goldberg score in PRP-treated group, resulting in reduced synovial hypertrophy and leukocyte infiltration. Recovery of safranin and type II collagen staining with PRP. PRP reduced IL-6 and VEGF staining within cartilage and synovium in PRP-treated joints. Reduction in IL-6, VEGF, IGF-1, and IL-1 concentration after injection of PRP to control levels.

(continued)

Table 1. Continued

Study	PRP Cytologic Findings	Study Design	Results
Wu et al.[39] 2011	N/A	Human chondrocytes cultured with and without a collagen matrix and with and without PRP. Inflammatory environment created with addition of IL-1β and TNF-α.	Cell viability increased in a dose-dependent fashion from PRP administration. Cell proliferation reduction as a result of IL-1β, and TNF-α inhibited by addition of PRP. Inhibition of *SOX9*, type II collagen, and aggrecan expression by IL-1β and TNF-α was reduced by PRP. PRP inhibited expression of *COX-2* and *MMP-2*. Inhibitory effect of IL-1β and TNF-α on proteoglycan and type II collagen deposition reduced by PRP.
Kon et al.[40] 2010	Mean platelet concentration in whole blood: 281 ± 56 × 10^3 /μL Mean platelet concentration in PRP: 874 ± 87 × 10^3/μL	Osteochondral lesions in sheep (n = 12). Bilateral osteochondral lesion created in femoral condyles. Three treatment groups: empty defect, biomimetic scaffold, biomimetic scaffold + PRP. Euthanized at 6 mo postoperatively.	Incomplete bone defect healing and more irregular bone-cartilage surface in PRP + scaffold compared with scaffold alone. Microradiographic evaluation showed poorer bone regeneration in PRP + scaffold compared with scaffold alone. Poorer histologic scores and type II collagen deposition in PRP + scaffold compared with scaffold alone.
Bendinelli et al.[41] 2010	On average, 8-fold increase in platelet concentration	Human chondrocytes cultured with NF-κβ to stimulate inflammatory environment. PRP added to culture to examine inhibitory effects on NF-κβ.	*COX2* and *CXCR4* activity decreased by elevated HGF concentrations in PRP.
Milano et al.[42] 2010	Mean platelet concentration in whole blood: 351 ± 64 × 10^3/mL Mean platelet concentration in PRP: 1,415 ± 164 × 10^3/mL	Osteochondral lesions in sheep (n = 15). Eight-millimeter osteochondral lesion created in right stifle joint and allowed to mature over 12 mo. Reoperation at 12 mo and animals divided into 3 groups: microfracture alone, microfracture with PRP gel, microfracture with PRP liquid. Animals euthanized 6 mo postoperatively.	PRP gel showed best macroscopic healing, followed by PRP liquid. Microfracture alone showed poor lesion healing. Mean stiffness of PRP gel–treated lesion approximated normal. Both PRP gel and PRP liquid groups had significantly better histologic scores than group treated by microfracture alone.
Spreafico et al.[43] 2009	Mean platelet concentration in whole blood: 234 ± 46.8 × 10^3/μL Mean platelet concentration in PRP: 1,460 ± 292 × 10^3/μL	Human articular chondrocytes cultured in monolayer with fetal calf serum, human serum, or 1%, 5%, and 10% PRP or platelet-poor plasma. Culture assessed at 2, 9, and 20 d. Chondrocytes also cultured on 3D fibrin scaffold with 10% fetal calf serum, PRP, or platelet-poor plasma.	PRP dose-dependent increase in chondrocyte proliferation. Increase in *SOX9* and aggrecan gene expression in PRP-treated cultures. Increase in type II collagen transcription in PRP-treated cultures. Increase in proteoglycan deposition in PRP-treated cultures. No difference in histologic structure between treatment groups, but increase in proteoglycan and type II collagen deposition.
Qi et al.[44] 2009	Mean platelet concentration in whole blood: 2.44 ± 0.46 × 10^5/μL Mean platelet concentration in PRP: 18.25 ± 1.21 × 10^5/μL	Osteochondral lesion in rabbits (n = 38). Full-thickness cartilage lesions created in 33 rabbit knees, with treatment divided into 3 groups: untreated, bilayer collagen matrix, bilayer collagen matrix + PRP. Five knees underwent sham surgery with no defect creation to function as controls. Animals euthanized at 6 and 12 wk postoperatively.	Improved ICRS score in PRP + collagen matrix–treated group compared with other 2 groups. Increased proteoglycan content in PRP + collagen matrix–treated group. No difference in biomechanical stiffness between collagen matrix alone group and collagen matrix + PRP group.

(continued)

Table 1. Continued

Study	PRP Cytologic Findings	Study Design	Results
Saito et al.[45] 2009	Mean platelet concentration in whole blood: 27.5 ± 3.8 × 10^4/μL Mean platelet concentration in PRP: 1,081.0 ± 149.9 × 10^4/μL	Rabbit chondrocytes and rabbit osteoarthritis model (n = 33). Rabbit chondrocytes cultured in alginate beads with FBS, PRP, or PPP for 3 and 7 d. Microspheres impregnated with PRP injected into healthy rabbit knee joints for PCR assay for ECM gene expression. Osteoarthritis model created by transecting anterior cruciate ligament and then treated with injections of PBS alone, PBS-containing microspheres, PRP alone, and PRP-containing microspheres at 4 and 7 wk postoperatively.	Significantly increased glycosaminoglycan production from chondrocytes cultured in PRP compared with PPP and FBS. Increased levels of proteoglycan core protein mRNA in PRP-injected healthy rabbit joints. Both PRP alone and PRP + microspheres improved gross histologic scoring, with PRP + microspheres showing the greatest positive effect. Improved modified Mankin score of groups treated with PRP + microspheres and PRP-treated group.
Pettersson et al.[46] 2009	N/A	Human articular chondrocyte–seeded gelatin microcarriers embedded and cultured in a static in vitro model with 4 different adjuncts: whole blood, PRP, PPP, fibrin glue. Cultured for 4, 8, 12, and 16 wk.	No statistically significant difference in histologic characteristics or proteoglycan deposition between the 4 groups.
Sun et al.[47] 2010	Mean platelet concentration in whole blood: 24.53 × 10^4/μL Mean platelet concentration in PRP: 125.59 × 10^4/μL	Osteochondral lesion in rabbits (n = 24). Sixteen rabbits underwent bilateral surgery to create an osteochondral lesion, which was then treated with either a PLGA scaffold or PLGA scaffold + PRP. Eight rabbits underwent bilateral surgery for osteochondral lesion creation; however no treatment was administered. Animals euthanized at 4 and 12 wk postoperatively.	Significantly improved modified O'Driscoll scores in PLGA + PRP–treated group. Increased proteoglycan deposition in PLGA + PRP– treated group. Increased type II collagen deposition in PLGA + PRP– treated group. Increased subchondral bone repair in PLGA + PRP–treated group as assessed by micro-CT.
Mishra et al.[48] 2009	PRP platelet concentration 700% higher than baseline	Human mesenchymal stem cells cultured in 10% PRP or 10% FBS for 21 d.	PRP increased cell proliferation. PRP increased expression of both osteogenic and chondrogenic markers; however much more significant increase in *SOX9* and aggrecan (chondrogenic markers).
Zaky et al.[49] 2008	PRP platelet concentration range: 1-1.8 million/μL	Human bone marrow stromal cells cultured in 3 different mediums: FBS + FGF, FBS + 5% PRP, and 5% PRP alone. Cultured for 14 d.	PRP culture showed increased cell proliferation. Increased chondrogenic differentiation observed in PRP culture.
Drengk et al.[52] 2009	N/A	Sheep chondrocytes and mesenchymal stem cells cultured in 6 different cultures: 3D pellet culture system, 3D pellet culture system + PRP, cells suspended in fibrin clot, cells suspended in PRP clot, cells growing in monolayer, cells growing in monolayer + PRP.	PRP increased cell proliferation of both chondrocytes and MSCs. Decrease in immunohistochemical staining and mRNA expression by chondrocytes cultured with PRP.
Akeda et al.[50] 2006	Mean platelet concentration in whole blood: 359 ± 83 × 10^3/mL Mean platelet concentration in PRP: 1,399 ± 174 × 10^3/mL	Porcine chondrocytes cultured for 72 h in 3 different mediums: SBF, SBF + PRP, SBF + PPP.	PRP increased chondrocyte cellular proliferation. PRP increased proteoglycan synthesis and total proteoglycan content. PRP increased type II collagen synthesis.

(continued)

Table 1. Continued

Study	PRP Cytologic Findings	Study Design	Results
Brehm et al.[51] 2006	N/A	Osteochondral lesions in goats (n = 9). Two-stage procedure, first to harvest chondrocytes to engineer a cartilage construct, with a second procedure to implant the construct into defect. Four groups: implant + PRP, implant + periosteal flap + fibrin glue, implant + periosteal flap + PRP, implant + periosteal flap. Animals euthanized at 8 wk.	Gross appearance ICRS scores were improved in PRP + periosteal flap compared with periosteal flap alone. Mean histologic score was worse in PRP + periosteal flap compared with periosteal flap alone.

3D, 3-dimensional; BMP, bone morphogenetic protein; CT, computed tomography; ECM, extracellular matrix; FBS, fetal bovine serum; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; ICRS, International Cartilage Repair Society; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; IL-6, interleukin-6; N/A, not available; NF-κβ = nuclear factor—kappa beta; PBS, phosphate buffered saline; PCR, polymerase chain reaction; PLGA, poly(lactic-co-glycolic acid); PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell; SBF, serum free medium; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; WBC, white blood cell.

In Vitro Studies

Of the in vitro studies (Table 3), 9 examined the effect of PRP on chondrocytes (6 human, 2 rabbit, 1 porcine), 3 examined the effect on human mesenchymal stem cells (MSCs), and 1 examined the effect on combined chondrocytes and MSCs from sheep.

Three studies reported the effect of PRP on chondrocyte viability in vitro, with 2 concluding that PRP increases cell viability (66%)[36,39] and the other stating that PRP had no significant effect.[34] The effect of PRP on cellular proliferation of both chondrocytes and MSCs was also assessed by 7 studies.[36,39,43,48-50,52] Seven studies (100%) determined that PRP causes a statistically significant increase in cellular proliferation.

The influence of PRP on the synthetic capability of chondrocytes and MSCs was investigated in 7 studies.[35,36,43,45,46,50,52] Of these studies, 5 (71%) found that PRP had induced statistically significant increases in proteoglycan, glycosaminoglycan, and type II collagen deposition.[35,36,43,45,50] One study (14%) reported that PRP had no effect on proteoglycan synthesis.[46] One additional study (14%) found that chondrocytes cultured with PRP show decreased type II collagen production.[52]

Gene expression was an outcome assessment in 3 studies. Two studies determined that PRP increased expression of genes related to the synthetic capability of chondrocytes, with upregulation of *aggrecan*, *TGFB*, bone morphogenetic protein, and *SOX9*.[36,43] One study found that PRP decreased collagen II mRNA expression in chondrocytes.[52]

A single article reported the effect of PRP on migration of human subchondral progenitor cells and found that PRP increased migration.[35] The influence of PRP on the differentiation of MSCs was also assessed by 3 studies. Each of these studies concluded that PRP increased chondrogenic differentiation.[35,48,49]

Three studies examined the effect of PRP on chondrocytes cultured in a simulated inflammatory environment. An inflammatory environment was created by culturing chondrocytes in the presence of interleukin (IL)-1β, tumor necrosis factor (TNF)-α plus IL-1β, or nuclear factor—kappa beta (NF-κβ).[37,39,41] The first of these studies found that PRP partially decreased the inhibitory effect of IL-1β on type II collagen and aggrecan gene expression in chondrocytes. In addition, PRP lessened the increase in *ADAMTS* and *PTGS2* expression caused by IL-1β.[37] Wu et al.[39] assessed the effect of PRP on chondrocytes cultured with IL-1β and TNF-α and concluded that PRP prevented the inhibition of *COX9*, type II collagen, and aggrecan expression, resulting in a moderate increase in proteoglycan and type II collagen deposition. The third article simulated an inflammatory environment by culturing chondrocytes with NF-κβ and found that PRP decreased the inflammatory-mediated increase in *COX2* and *CXCR4* activity.[41]

In Vivo Studies

For the in vivo studies (Table 4), multiple animal models were used, including rabbit (4), sheep (3), pig (1), and

Table 2. Reporting on Cytologic Analysis of PRP

Component	Reported Studies (No.)	Studies Not Reporting (No.)
Platelet	15	6
White blood cells	2	19
Red blood cells	1	20

PRP, platelet-rich plasma.

Table 3. Results Reported In Vitro

Outcome	Significant Increase (No.)	No Significant Change (No.)	Significant Decrease (No.)
Cell viability	2	0	0
Cell proliferation	7	0	0
Proteoglycan and type II collagen content	5	1	1
Gene expression	2	0	1
Cell migration	1	0	0
Cell differentiation	3	0	0
Inflammatory mediation	3	0	0

Table 4. Results Reported In Vivo

Outcome	Significant Increase (No.)	No Significant Change (No.)	Significant Decrease (No.)
Cell viability	1	0	0
Gene expression	2	0	0
Gross appearance of cartilage repair	3	0	1
Histologic assessment of cartilage repair	4	0	2
Proteoglycan content	2	0	0
Collagen type II deposition	2	0	1
Cartilage stiffness	2	0	0
Inflammatory mediation	1	0	0

goat (1). Seven of the studies used PRP as an adjunct to the treatment of a focal cartilage lesion.[32,33,40,42,44,47,51] One study used PRP as a primary management strategy for an inflammatory arthritis model,[38] whereas another study used it in an osteoarthritis model.[45]

Cartilage Repair. Six studies described the effect of PRP on the histologic appearance of the articular cartilage repair. Four (66.7%) concluded that PRP improved the histologic appearance of the cartilage,[33,42,44,45,47] whereas 2 reports stated that it worsened the histologic scores when compared with not using PRP.[40,51] Gross appearance of articular cartilage was assessed by 4 studies, with 3 (75%) concluding that PRP improved the gross appearance,[33,42,51] and one reporting that PRP worsened the gross appearance.[40]

Proteoglycan content of cartilage repair tissue was assessed histologically in 2 studies, with each reporting that PRP increased the proteoglycan content of the cartilage tissue.[44,45,47] Additionally, type II collagen content of cartilage repair tissue was measured by 3 studies, with 2 (66.6%) concluding that type II collagen content was increased with the administration of PRP.[38,47] One study stated that PRP decreased type II collagen content of cartilage repair tissue.[40]

The effect of PRP on the stiffness of the cartilage repair was measured by 2 studies, both of which determined that PRP increased cartilage stiffness.[33,42] A single article studied the influence of the PRP on gene expression, finding that PRP increased expression of *SOX9*, *CB2*, *CHM1*, and aggrecan genes.[32]

Table 5. Analysis of Platelet-Rich Plasma

Required concentrations to be reported	Platelet, white blood cell, red blood cell
Optional concentrations to be reported	Growth factors: including but not limited to TGF-β, PDGF, and VEGF Cytokines: including but not limited to IL-1, IL-6, TNF, IRAP

IL, interleukin; IRAP, interleukin receptor antagonist protein; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Osteoarthritis and Inflammatory Arthritis. One study assessed the effect of PRP in an osteoarthritis model, evaluating the intra-articular proteoglycan core mRNA level, as well as the gross and histologic appearance of the articular cartilage. The authors concluded that PRP increased the proteoglycan core mRNA levels and improved the gross and histologic appearance of the cartilage.[45]

The effect of PRP in an inflammatory arthritis model was also assessed by one study. The article reported the histologic appearance of the synovium; histologic staining results for proteoglycans, type II collagen, IL-6, and vascular endothelial growth factor (VEGF); and multiplexing assay results for IL-6, VEGF, and IL-1. PRP was found to reduce synovial hypertrophy, increase proteoglycan and type II collagen staining, decrease IL-6 and VEGF staining, and reduce IL-6, VEGF, insulin-like growth factor (IGF)-1, and IL-1 intra-articular levels as measured by multiplex assay.[38]

Discussion

The in vitro and in vivo basic science literature indicates that PRP has several potential effects when used in an investigational setting as an adjunct for cartilage repair or for the conservative management of osteoarthritis. Most studies (71%) concluded that PRP increases the synthetic capacity of chondrocytes, including upregulation of gene expression, proteoglycan production, and deposition of type II collagen.[36,43-45,47,50] Increasing the proteoglycan and type II collagen content in repair tissue may be of benefit in bone marrow–stimulation techniques (e.g., microfracture). The tissue infill after bone marrow stimulation has been shown to be composed of primarily type I collagen and poorer proteoglycan and type II collagen content than found in normal cartilage.[53-57] Therefore, the fibrous repair tissue shows inherently poorer biological and mechanical properties than does hyaline cartilage, causing possible degeneration over time.[53] The basic science evidence indicates that PRP may induce a more hyaline-like repair tissue after bone marrow stimulation procedures by increasing proteoglycan and type II collagen deposition by chondrocytes. However, the in vitro consensus is not unanimous; Pettersson et al.[46] found that PRP did not increase proteoglycan content and Drengk et al.[52] found that PRP decreased collagen type II deposition by chondrocytes.

Increases in chondrocyte viability[32,36,39] and proliferation[36,39,43,50,52] resulting from PRP may also prove beneficial in cartilage repair. Decreases in chondrocyte viability, which have been shown after osteochondral autograft implantation[58] secondary to storage of osteochondral allografts,[59] have the theoretical potential to be countered by the effects of PRP. Additionally, increasing chondrocyte proliferation may benefit techniques that involve the implantation of autologous chondrocytes.[60]

PRP may influence cartilage repair after bone marrow stimulation through its potential effect on MSCs.[35,48,49,52] With the purpose of bone marrow stimulation being to breach the subchondral plate, thus stimulating an inflammatory response and migration of subchondral-derived MSCs, an effect of PRP on MSCs may influence the resulting quality of cartilaginous repair tissue. In this regard, Krüger et al.[35] found that PRP increases the migration and chondrogenic differentiation of MSCs. Additionally, there is also evidence indicating that PRP increases the proliferation and synthetic capability of MSCs.[35,48,49,52] The potential influences of PRP on MSCs are not limited to bone marrow stimulation; they may also be translated to novel cartilage repair strategies, including autologous culture–expanded mesenchymal stem cell implantation and autologous matrix–induced chondrogenesis.[61,62]

The catabolic intra-articular environment existing in the presence of pathologic processes of cartilage may also be influenced by the administration of PRP. The presence of catabolic cytokines (e.g., IL-1β and TNF-α) increases the activity and levels of matrix metalloproteinases, ADAMTS, and elastases by both synovial cells and chondrocytes. This may then potentially cause progression of osteoarthritis and inhibition of production of regenerative tissue in cartilage repair.[63,64] Interestingly, the in vitro literature reports that PRP partially inhibits the catabolic effects of IL-1β, TNF-α, and NF-κβ on chondrocytes.[37,39,41] Van Buul et al.[37] found that PRP decreased IL-1β–mediated inhibition of type II collagen and aggrecan gene expression and also decreased the IL-1β–induced increase of *ADAMTS4* and *PTGS2* gene expression. This has been extrapolated to an in vivo inflammatory arthritis model in pigs by Lippross et al.[38] The investigators found that the administration of PRP caused a moderate recovery of type II collagen lost to inflammation and also reduced IL-1β levels, synovial hypertrophy, and leukocyte infiltration.

The in vitro findings have been somewhat corroborated in the in vivo models using a variety of modalities for cartilage repair. Milano et al.,[33,42] in 2 separate studies, reported improved gross and histologic findings, as well as increased type II collagen deposition, when using PRP as an adjunct to microfracture surgery for osteochondral lesions. Similar results have been described when using PRP in conjunction with a poly (lactic-co-glycolic acid) (PLGA) scaffold,[47] collagen matrix,[44] and autologous chondrocyte implantation.[32] However, not all studies have reported a favorable outcome as a result of the administration of PRP. In a sheep osteochondral lesion model treated with a biomimetic scaffold, Kon et al.,[40] concluded that the addition of PRP resulted in a poorer histologic outcome. This may be attributed to the chemical composition of the implant, because differing biomaterials have been shown to affect the activation of platelets.[65-67]

Limitations

A key limitation of this review is that most of the studies failed to adequately characterize the contents of the PRP used, with only one report (4.8%) describing the platelet, WBC, and RBC counts.[37] Furthermore, 6 studies (28.6%) failed to make any mention of the platelet concentration of the PRP.[32,34,39,46,51,52] Due to this lack of reporting, it was not possible to draw conclusions on the effect of the varying concentrations of the constituents of PRP. Additionally, Castillo et al.[68] found that the contents of PRP vary significantly depending on the centrifugation system used. Compounding this issue, in a study by Mazzocca et al.[69] the authors drew blood from 8 healthy individuals at 3 different time points, and after centrifugation to produce PRP, they measured the concentration of platelets, WBCs, and growth factors. The results showed that the levels of the bioactive components in PRP show both inter- and intra-individual variability. Therefore, when investigating the effects of PRP, it is not sufficient to simply describe the preparation method and label the blood product as PRP.[70] To compare the results of different studies assessing the use of PRP for any orthopaedic indication, a basic cytologic analysis of PRP is a necessary requirement (Table 5).

The limitations inherent to in vivo animal studies, and thus this systematic review, are numerous and often specific to the type of animal model used. First, cartilage lesions created in animal models (e.g., rabbits) are smaller than the size of the commonly treated lesion in humans.[71] Additionally, joint loading conditions differ between humans and animals because of varying cartilage thickness and anatomic differences. As an example, the rabbit, which was used in 4 of the studies included in this systematic review, uses the trochlear groove as a partial weight-bearing surface because of the flexed position of the knee and has a cartilage thickness of 0.3 ± 0.07 mm at the medial femoral condyle.[72] Furthermore, and specifically relevant to the study of PRP, the physiologic parameters and cytologic characteristics of blood and platelets may differ between animals and humans.[71] As a result of these limitations, it is challenging to extrapolate the results to clinical practice. Nevertheless, it is accepted that in vivo research is useful to investigate the proof of concept for experimental treatments, and therefore well-designed clinical trials are needed to translate this evidence to clinical use.

Conclusions

The current basic science evidence suggests that PRP has several potential effects on cartilage repair and osteoarthritis, and proof of concept has been established. Well-designed RCTs are needed to extrapolate this evidence to the clinical setting.

References

1. Miller Y, Bachowski G, Benjamin R, Eklund D, Hibbard A, Lightfoot T. *Practice guideline for blood transfusion: A compilation from recent peer-reviewed literature.* Ed 2. Washington, DC: American Red Cross, 2012;56.
2. Smyth S, McEver R, Weyrich A, et al. Platelet functions beyond hemostasis. *J Thromb Haemost* 2009;7:1759-1766.
3. de Vos RJ, Weir A, van Schie HT, et al. Platelet-rich plasma injection for chronic Achilles tendinopathy: A randomized controlled trial. *JAMA* 2010;303:144-149.
4. Nin JR, Gasque GM, Azcarate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy* 2009;25:1206-1213.
5. Kalaci A, Cakici H, Hapa O, Yanat A, Dogramaci Y, Sevinc T. Treatment of plantar fasciitis using four different local injection modalities: A randomized prospective clinical trial. *J Am Podiatr Med Assoc* 2009;99:109-113.
6. Sys J, Weyler J, van der Zijden T, Parizel P, Michielsen J. Platelet-rich plasma in mono-segmental posterior lumbar interbody fusion. *Eur Spine J* 2011;20:1650-1657.
7. Dallari D, Savarino L, Stagni C, et al. Enhanced tibial osteotomy healing with use of bone grafts supplemented with platelet gel or platelet gel and bone marrow stromal cells. *J Bone Joint Surg Am* 2007;89:2413-2420.
8. Castricini R, Longo U, de Benedetto M, et al. Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: A randomized controlled trial. *Am J Sports Med* 2011;39: 258-265.
9. Peerbooms J, de Wolf G, Colaris J, Bruijn D, Verhaar J. No positive effect of autologous platelet gel after total knee arthroplasty. *Acta Orthop* 2009;80:557-562.
10. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med* 2006;34:1774-1778.
11. Kon E, Mandelbaum B, Buda R, 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: 1499-1501.
12. Mei-Dan O, Carmont M, Laver L, Mann G, Maffulli N, Nyska M. Platelet-rich plasma or hyaluronate in the management of osteochondral lesions of the talus. *Am J Sports Med* 2012;40:534-541.
13. Thanasas C, Papadimitriou G, Charalambidis C, Paraskevopoulos I, Papanikolaou A. Platelet-rich plasma versus autologous whole blood for the treatment of chronic lateral elbow epicondylitis: A randomized controlled clinical trial. *Am J Sports Med* 2011;39: 2130-2134.
14. Gosens T, Peerbooms JC, van Laar W, den Oudsten BL. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: A double-blind randomized controlled trial with 2-year follow-up. *Am J Sports Med* 2011;39:1200-1208.
15. Sheth U, Simunovic N, Klein G, et al. Efficacy of autologous platelet-rich plasma use for orthopaedic indications: A meta-analysis. *J Bone Joint Surg Am* 2012;94:298-307.
16. Aigner T, Stove J. Collagens: Major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. *Adv Drug Deliv Rev* 2003;55:1569-1593.
17. Mankin H. The response of articular cartilage to mechanical injury. *J Bone Joint Surg Am* 1986;64:460-466.
18. Jackson D, Lalor P, Aberman H, Simon T. Spontaneous repair of full thickness defects of articular cartilage in a goat model: A preliminary study. *J Bone Joint Surg Am* 2001;83:53-64.
19. O'Loughlin P, Heyworth B, Kennedy JG. Current concepts in the diagnosis and treatment of osteochondral lesions of the ankle. *Am J Sports Med* 2010;32:392-404.
20. Farr J, Cole BJ, Dhawan A, Kercher J, Sherman S. Clinical cartilage restoration: Evolution and overview. *Clin Orthop Relat Res* 2011;469:2696-2705.
21. Hunt S, Sherman O. Arthroscopic treatment of osteochondral lesions of the talus with correlation of outcome scoring systems. *Arthroscopy* 2003;19:360-367.
22. Murawski C, Foo L, Kennedy J. A review of arthroscopic bone marrow stimulation techniques of the talus: the good, the bad, and the causes for concern. *Cartilage* 2010;1:137-144.
23. Bedi A, Feeley BT, Williams RJ III. Management of articular cartilage defects of the knee. *J Bone Joint Surg Am* 2010;92:994-1009.
24. Knutsen G, Engebretsen L, Ludvigsen T, et al. Autologous chondrocyte implantation compared with microfracture in the knee. *J Bone Joint Surg Am* 2004;86:455-464.
25. Lee K, Bai L, Yoon T, Jung S, Seon J. Second-look arthroscopic findings and clinical outcomes after microfracture for osteochondral lesions of the talus. *Am J Sports Med* 2009;37:63S-70S.
26. Valderrabano V, Leumann A, Rasch H, Egelhof T, Hintermann B, Pagenstert G. Knee-to-ankle mosaicplasty for the treatment of osteochondral lesions of the ankle joint. *Am J Sports Med* 2009;37:105S-111S.
27. Becher C, Driessen A, Hess T, Longo U, Maffuli N, Thermann H. Microfracture for chondral defects of the talus: Maintenance of early results at midterm follow-up. *Knee Surg Sports Traumatol Arthrosc* 2010;18: 656-663.
28. Fortier LA, Barker JU, Strauss EJ, McCarrel TM, Cole BJ. The role of growth factors in cartilage repair. *Clin Orthop Relat Res* 2011;469:2706-2715.
29. Smyth NA, Fansa AM, Murawski CD, Kennedy JG. Platelet-rich plasma as a biological adjunct to the surgical treatment of osteochondral lesions of the talus. *Tech Foot Ankle Surg* 2012;11:18-25.
30. Spakova T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil* 2012;91:411-417.
31. Filardo G, Kon E, Ruiz MP, et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: Single- versus double-spinning approach. *Knee Surg Sports Traumatol Arthrosc* 2012;20:2082-2091.
32. Lee H, Park KM, Park KD, Joung YK, Do Hee S. Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. *J Control Release* 2012;159: 332-337.

33. Milano G, Deriu L, Passino ES, et al. Repeated platelet concentrate injections enhance reparative response of microfractures in the treatment of chondral defects of the knee: An experimental study in an animal model. *Arthroscopy* 2012;28:688-701.
34. Sitek P, Wysocka-Wycisk A, Kępski F, Król D, Bursig H, Dyląg S. PRP-fibrinogen gel-like chondrocyte carrier stabilized by TXA-preliminary study. *Cell Tissue Bank* 2013;14:133-140.
35. Krüger 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;30:845-852.
36. 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.
37. van Buul GM, Koevoet WLM, et al. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports Med* 2011;39:2362-2370.
38. Lippross S, Moeller B, Haas H, et al. Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint. *Arthritis Rheum* 2011;63:3344-3353.
39. Wu C, Chen W, Zao Bin, et al. Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokine-inhibited chondrogenesis. *Biomaterials* 2011;32:5847-5854.
40. Kon E, Filardo G, Delcogliano M, et al. Platelet autologous growth factors decrease the osteochondral regeneration capability of a collagen-hydroxyapatite scaffold in a sheep model. *BMC Musculoskelet Disord* 2010;11:220.
41. Bendinelli P, Matteucci E, Dogliotti G, et al. Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-κB inhibition via HGF. *J Cell Physiol* 2010;225:757-766.
42. Milano G, Passino ES, Deriu L, et al. The effect of platelet rich plasma combined with microfractures on the treatment of chondral defects: An experimental study in a sheep model. *Osteoarthritis Cartilage* 2010;18:971-980.
43. Spreafico A, Chellini F, Frediani B, et al. Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. *J Cell Biochem* 2009;108: 1153-1165.
44. Qi YY, Chen X, Jiang YZ, et al. Local delivery of autologous platelet in collagen matrix simulated in situ articular cartilage repair. *Cell Transplant* 2009;18:1161-1169.
45. Saito M, Takahashi K, Arai Y, et al. Intraarticular administration of platelet-rich plasma with biodegradable gelatin hydrogel microspheres prevents osteoarthritis progression in the rabbit knee. *Clin Exp Rheumatol* 2009;27:201-207.
46. Pettersson S, Wetterö J, Tengvall P, Kratz G. Human articular chondrocytes on macroporous gelatin microcarriers form structurally stable constructs with blood-derived biological glues. *J Tissue Eng Regen Med* 2009;3: 450-460.
47. 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: 589-597.
48. Mishra A, Tummala P, King A, et al. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. *Tissue Eng Part C Methods* 2009;15:431-435.
49. Zaky SH, Ottonello A, Strada P, Cancedda R, Mastrogiacomo M. Platelet lysate favours expansion of human bone marrow stromal cells for bone and cartilage engineering. *J Tissue Eng Regen Med* 2008;2:472-481.
50. Akeda K, An H, Okuma M, et al. Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. *Osteoarthritis Cartilage* 2006;14: 1272-1280.
51. Brehm W, Aklin B, Yamashita T, et al. Repair of superficial osteochondral defects with an autologous scaffold-free cartilage construct in a caprine model: Implantation method and short-term results. *Osteoarthritis Cartilage* 2006;14:1214-1226.
52. Drengk A, Zapf A, Sturmer EK, Sturmer KM, Frosch KH. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells. *Cells Tissues Organs* 2009;189: 317-326.
53. Furukawa T, Eyre DR, Koide S, Glimcher MJ. Biochemical studies on repair cartilage resurfacing experimental defects in the rabbit knee. *J Bone Joint Surg Am* 1980;62: 79-89.
54. Hjertquist S, Lemperg R. Histological, autoradiographic and microchemical studies of spontaneously healing osteochondral articular defects in adult rabbits. *Calcif Tissue Res* 1971;8:54-72.
55. Shapiro F, Koide S, Glimcher M. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1993;75:532-553.
56. Mitchell N, Shepard N. The resurfacing of adult rabbit articular cartilage by multiple perforations through the subchondral bone. *J Bone Joint Surg Am* 1976;58: 230-233.
57. Frisbie DD, Oxford JT, Southwood L, et al. Early events in cartilage repair after subchondral bone microfracture. *Clin Orthop Relat Res* 2003;407:215-227.
58. Gulotta LV, Rudzki JR, Kovacevic D, Chen CC, Milentijevic D, Williams RJ III. Chondrocyte death and cartilage degradation after autologous osteochondral transplantation surgery in a rabbit model. *Am J Sports Med* 2009;37:1324-1333.
59. William S, Amiel D, Ball S, et al. Prolonged storage effects on the articular cartilage of fresh human osteochondral allografts. *J Bone Joint Surg Am* 2003;85: 2111-2120.
60. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;331:889-895.
61. Wakitani S, Goto T, Pineda S, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1994;76:579-592.
62. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: An observational cohort study. *Am J Sports Med* 2010;38: 1110-1116.

63. Sindrilaru A, Peters T, Wieschalka S, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *J Clin Invest* 2011;121:985-997.
64. Song R, Tortorella M, Malfait A. Aggrecan degradation in human articular cartilage explants is mediated by both ADAMTS-4 and ADAMTS-5. *Arthritis Rheum* 2007;56: 575-585.
65. Rolden J, Jepsen S, Miller J, et al. Bone formation in the presence of platelet-rich plasma vs bone morphogenetic protein-7. *Bone* 2004;34:80-90.
66. Niemer P, Lunginbuhl R, Szalay K. The effect of platelet-rich plasma on healing in critical-size long-bone defects. *Biomaterials* 2008;29:3983-3992.
67. Wiltfand J, Schlegel K, Schultze-Mosgan S, Nkenke E, Zimmermann R, Kessler P. Sinous floor augmentation with beta-tricalcium phosphate: does platelet rich plasma promote its osseous integration and degradation? *Clin Oral Implants Res* 2003;14:213-218.
68. Castillo T, Pouliot M, Kim H, Dragoo J. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation system. *Am J Sports Med* 2011;39:266-271.
69. Mazzocca A, McCarthy M, Chowaniec D, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am* 2012;94:308-316.
70. Boswell SG, Cole BJ, Sundman EA, et al. Platelet-rich plasma: A milieu of bioactive factors. *Arthroscopy* 2012;28:429-439.
71. Chu CR, Szczodry M, Bruno S. Animal models for cartilage regeneration and repair. *Tissue Eng Part B Rev* 2010;16:105-115.
72. Ahern BJ, Parvizi J, Boston R, Schaer TP. Preclinical animal models in single site cartilage defect testing: A systematic review. *Osteoarthritis Cartilage* 2009;17:705-713.
73. Moher D, Liberati A, Tetzlaff J, Altman DG. The PRISMA Group. Preferred reporting items for systematic reviews and meta- analyses: The PRISMA statement. *PLoS Med* 2009;6:e1000097.