# The Efficacy of Platelet-Rich Plasma for Ligament Injuries

## A Systematic Review of Basic Science Literature With Protocol Quality Assessment

Kyle N. Kunze,*† MD, Jeevana J. Pakanati,‡ MS, Amar S. Vadhera,§ BS, Evan M. Polce,‖ BS, Brady T. Williams,¶ MD, Kevin C. Parvaresh,§ MD, and Jorge Chahla,§ MD, PhD

*Investigation performed at Rush University Medical Center, Chicago, Illinois, USA*

**Background:** Despite the existence of many clinical studies on platelet-rich plasma (PRP) interventions for ligamentous pathology, basic science consensus regarding the indications, mechanisms, and optimal composition of PRP for treating ligament injuries is lacking.

**Purpose:** To (1) compare the efficacy of PRP in animal models of ligament injury with placebo and (2) describe the potential variability in PRP preparation using accepted classification systems.

**Study Design:** Systematic review.

**Methods:** The Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, PubMed, Embase, and Ovid MEDLINE were queried in April 2020 for in vivo and in vitro basic science studies regarding PRP use for ligament injury. Study design, results, PRP composition, and analyzed cellular and molecular markers were extracted, and outcomes relative to control models were documented. Bias was assessed using the SYRCLE risk-of-bias tool.

**Results:** Included were 43 articles (31 in vivo and 12 in vitro studies) investigating the anterior cruciate ligament/cranial cruciate ligament (n = 32), medial collateral ligament (n = 6), suspensory ligament (n = 3), patellar ligament (n = 1), and Hock ligament (n = 1). Platelet concentration was reported in 34 studies (77.3%); leukocyte composition, in 12 (27.3%); and red blood cell counts, in 7 (15.9%). With PRP treatment, 5 of 12 in vitro studies demonstrated significant increases in cell viability, 6 of 12 in gene expression, 14 of 32 in vivo studies reported superior ligament repair via histological evaluation, and 13 in vivo studies reported superior mechanical properties. Variability in PRP preparation methods was observed across all articles, and only 1 study reported all necessary information to be classified by the 4 schemes we used to evaluate reporting. Among the in vivo studies, detection and performance bias were consistently high, whereas selection, attrition, reporting, and other biases were consistently low.

**Conclusion:** Conflicting data on the cellular and molecular effects of PRP for ligament injuries were observed secondary to the finding that included studies were heterogeneous, limiting interpretation across studies and the ability to draw meaningful conclusions. Clinical trials and any causal relationship between PRP use in ligament injuries and its potential for regeneration and healing should be pursued with caution if based solely on basic science data.

**Keywords:** platelet-rich plasma; PRP; ligaments; basic science; biologic; cytology

Injuries to ligaments, particularly the cruciate and collateral ligaments of the knee, remain a frequent occurrence and burden to the health care system. In a review of >25,000 reported injuries in high school athletes, knee injuries and ligament sprains were most commonly reported, with knee injuries most often involving the medial collateral ligament (MCL), followed by patellar tendon, anterior cruciate ligament (ACL), meniscus, lateral collateral ligament (LCL), and posterior cruciate ligament (PCL).[12] Both the high incidence of these injuries and patient expectations for expedited recovery and rehabilitation for such injuries have prompted investigations into adjunct therapies aimed to enhance ligament healing. As such, attention has been turned to biologics, as these adjuncts have demonstrated promising healing benefits for the treatment of various musculoskeletal injuries.[8,9,33]

Platelet-rich plasma (PRP) is used as a biologic agent to enhance tissue and ligament repair.[19,38,61] Its potential role

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in ligament healing has made it of great interest to the field of orthopaedic sports medicine, with early evidence suggesting that the combination of cellular and molecular components including platelets, leukocytes, growth factors, fibrinogen, erythrocytes, hormones, and proteases may reduce pain,[4] accelerate ligament repair, and expedite return to function.[8,9,21,34,53] However, some investigations have found minimal to no benefit of using PRP, specifically in applications to ligament healing after ACL reconstruction.[16,37,42,60]

One potential yet crucial explanation of the variability observed in clinical studies may be because of heterogeneous PRP compositions and inconsistent preparation methods, as these may lead to different effects.[7,24] Several recent studies have acknowledged that reporting standards for PRP are an essential component of investigating its use, as there is a paucity of evidence concerning the effects that changes in the composition and preparation of PRP may confer on its efficacy.[15,18,35,43] Because of this uncertainty, several recent guidelines for reporting on biologics have been created in order to minimize heterogeneity in reporting and biologic preparation. The Minimum Information for Studies Evaluating Biologics in Orthopaedics is one such set of reporting guidelines, consisting of 25 specific checklist items, that was created in order to guide appropriate use.[44] Several research journals have accordingly mandated that authors abide by these guidelines when submitting clinical trials investigating PRP use.

To more appropriately treat ligament pathology with PRP and develop comprehensive indications for its use, clinicians and researchers need a better understanding of how PRP influences ligamentous healing at a basic science level and how variations in PRP preparation may influence its efficacy. The purpose of the current study was to (1) compare the efficacy of PRP versus placebo in animal models of ligament injury and (2) assess the variability in PRP preparation using accepted classification systems. We hypothesized that PRP would confer changes beneficial to ligament healing at the molecular level and gross tissue level when compared with controls, including increased angiogenic markers, growth factor concentrations, and load to failure; furthermore, we proposed that the quality of the literature available would be low and that the lack of reporting on specifically the cytological characteristics of PRP would limit the interpretability of the literature.

## METHODS

### Article Search and Selection Process

A systematic review of the Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, PubMed, Embase, and Ovid MEDLINE was performed in April 2020 using PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines with a PRISMA checklist. The search was performed using the following Boolean search terms: (platelet-rich plasma OR PRP OR autologous conditioned plasma OR ACP) AND (ligament OR ligaments OR ACL OR MCL OR LCL OR PCL OR UCL OR anterior cruciate OR ulnar collateral OR medial collateral OR lateral collateral OR posterior cruciate).

The inclusion criteria for full-text review were in vivo and in vitro studies examining the effects of PRP on ligaments and fibroblasts derived from ligaments compared with a well-defined control group (saline solution, no treatment, or control cell medium) or any comparative group (varied concentrations of bone marrow aspirate, PRP, lyophilized platelet product, blood, platelet-poor plasma, autologous bone marrow, collagen-platelet composite, nonsteroidal anti-inflammatory drugs). Animal studies that observed the effects of PRP on traumatic and surgically created ligament injuries were included. Various preparations and formulations of PRP were included, such as single- and twice-centrifuged PRP, calcium chloride- and thrombin-activated PRP, PRP injections, PRP gels, and releasates from PRP clots. Exclusion criteria included non-English-language articles, clinical studies, randomized controlled trials, review articles, articles not published in peer-reviewed journals, and studies without a control or comparative group. All references within included studies were cross-referenced for potential inclusion. Two authors independently performed the search and identified studies for inclusion (E.M.P., B.T.W.). Articles were screened by title, then abstract, and finally full-text review. Discrepancies were resolved by consensus.

### Data Extraction

Data were extracted using a predefined data collection sheet. Data collected included the preparation methods and cytological characteristics of PRP, growth factor concentration, adhesive protein concentration, clotting factor concentration, fibrinolytic factors, proteases and antiproteases, basic proteins, membrane glycoproteins, dense granule bioactive molecules, proinflammatory cytokine concentration,

*Address correspondence to Kyle N. Kunze, MD, Department of Orthopaedic Surgery, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA (email: kylekunze7@gmail.com) (Twitter: @kylekunzemd).
†Department of Orthopaedic Surgery, Hospital for Special Surgery, New York, New York, USA.
‡Rosalind Franklin University, Chicago Medical School, Chicago, Illinois, USA.
§Department of Orthopaedic Surgery, Division of Sports Medicine, Rush University Medical Center, Chicago, Illinois, USA.
‖University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA.
¶Department of Orthopaedic Surgery, University of Colorado, Aurora, Colorado, USA.
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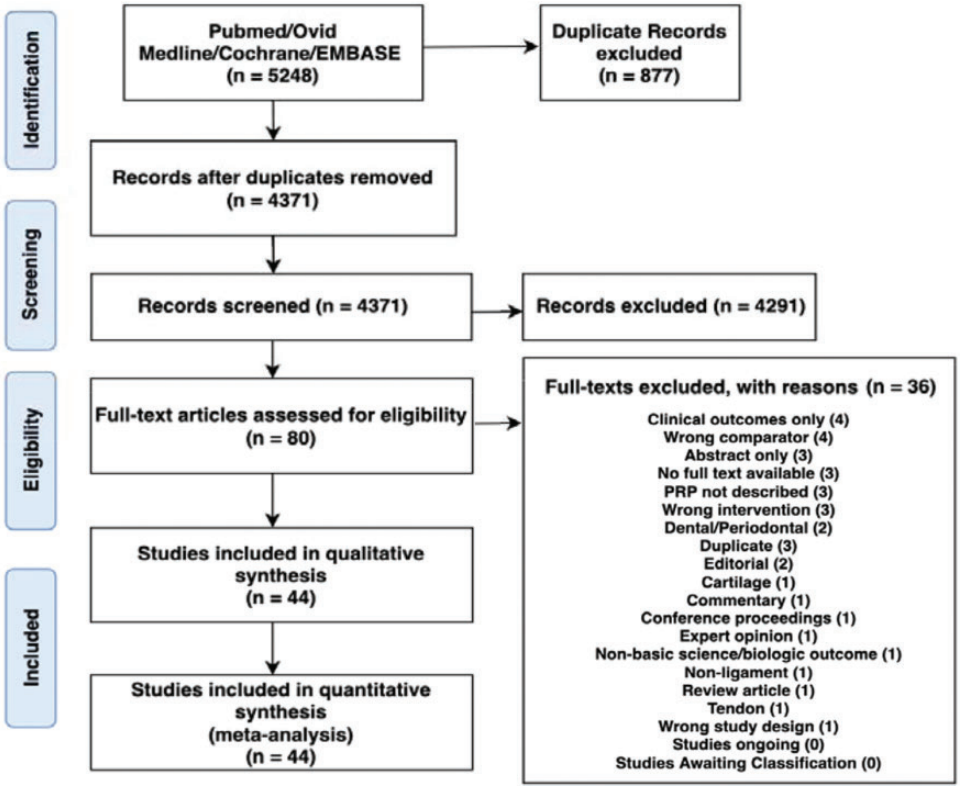


**Figure 1.** PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram representing the process of individual study inclusion after application of the study algorithm and each of the exclusion criteria. PPR, platelet-rich plasma.

anti-inflammatory cytokine concentration, and other proteins. In addition, study design and methods, study subjects, outcomes measured, and results were recorded. These variables included cell viability, cell proliferation, cell migration, gene expression, mechanical properties, gross appearance, proteoglycan and collagen content, and mechanical or histological examinations. If results were significantly different between the experimental and the comparative cohorts, they were considered to be positive or negative results based on the relative change observed. If no difference was observed between the experimental and comparative cohort groups, it was recorded as no change observed.

### Study Quality

To determine the quality and degree of variability of PRP preparation methods, each included study was assessed via 4 established PRP classification schemes: Magalon,[35] Erhenfest,[18] PAW,[15] and Sports Medicine.[43] Successful classification via 1 scheme was noted if all pertinent information was available for each aspect of the system. After data analysis was complete, data were analyzed for trends in outcomes, comparing PRP treatment with controls.

### Bias Assessment

The risk of bias of in vivo animal studies was assessed using the SYRCLE risk-of-bias tool.[28] This is a 10-item validated tool that assesses selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases based on the Cochrane risk-of-bias tool. This tool has been adjusted for aspects of bias that play a specific role in animal intervention studies. We were unable to identify a risk-of-bias assessment tool for in vitro studies, and therefore the risk of bias in these studies was not assessed. Bias was assessed by 2 independent authors (J.J.P. and A.S.V.), and discrepancies were resolved by a third author (K.N.K.).

### Statistical Analysis

Extracted data were described as frequencies with percentages when possible. Data not amenable to quantification were described narratively. Because PRP preparation methods, animal models, and outcomes were reported variably and differed among studies, data were inappropriate to examine via meta-analysis.

## RESULTS

A total of 5248 articles were identified via the initial search. Of these, 43 studies were included in the final analysis (Figure 1). A total of 31 were classified as in vivo, whereas 12 were classified as in vitro studies. Of these, the following ligaments were investigated: ACL/cranial cruciate ligament (CCL) (n = 32), MCL (n = 6), suspensory ligament

TABLE 1
Summary of Cytology Reporting in PRP Ligament Studies

| Component | Studies Reporting, n (%) | Studies Not Reporting, n (%) |
|---|---|---|
| Platelet count | 33 (76.4) | 10 (23.3) |
| White blood cell count | 12 (27.9) | 31 (72.1) |
| Red blood cell count | 7 (16.3) | 36 (83.7) |

PPR, platelet-rich plasma.

(n = 3), patellar ligament (n = 1), and Hock ligament (n = 1). The types of biologic markers reported as outcomes varied greatly across studies (Appendix Table A1)

## In Vitro Ligament Publications

*PRP Composition and Reporting Trends.* Of the 12 in vitro ligament studies, 7 studies examined ACL explant cells,[10,11,17,20,36,64,65] 3 studies examined suspensory ligament cells,[41,56,57] 1 study examined human anterior cruciate ligamentocytes,[30] and 1 study examined human periodontal ligament fibroblasts (Appendix Table A2).[51] Of the 7 studies that examined ACL explant cells, 4 studies assessed porcine ACL-derived fibroblasts,[10,11,64,65] whereas the remaining 3 studies evaluated human ACL fibroblasts.[17,20,36]

In 8 (66.7%) studies,[#] platelet concentration of the final PRP preparation was reported to be greater than that of whole blood, although variability was observed between all preparation protocols (Table 1). One study compared the platelet concentration prepared from autologous conditioned plasma (ACP) (Arthrex ACP Double Syringe System; Arthrex) versus the platelet concentration prepared from Biomet GPS III (GPS; Biomet) and reported the resultant platelet concentration from ACP was lower than platelet concentration made from GPS.[51]

A total of 4 (33.3%) studies reported leukocyte concentrations, of which 3 (25%) studies did not report whether leukocyte concentrations differed from the control group[5,10,47] and 1 study reported a relative decrease in leukocyte count.[48] Only 1 study used PRP that was classified as leukocyte-poor[6]; the remaining 3 studies did not have enough information provided to report.[8,41,42] One study compared the leukocyte concentrations of preparations using ACP and GPS, reporting that the leukocyte concentration of ACP preparations was lower than the leukocyte concentration of GPS.[51]

A total of 4 (33.3%) studies reported red blood cell (RBC) count in their PRP preparations. Of these, 1 study did not report whether the RBC count differed from that of the control,[62] whereas 3 (25%) studies noted that the RBC count decreased relative to the RBC count of the control.[5,30,48] Only 1 (8.3%) study reported a full cytology count.[30]

*Effects of PRP In Vitro.* In total, 5 studies reported that PRP increased cell viability when compared with a control group,[10,11,17,20,64] and 2 studies reported a significant increase in cell proliferation with PRP treatment.[30,36] Further, 3 studies reported a significant increase in collagen content with PRP treatment,[11,20,65] whereas 2 studies reported no difference between the PRP and control groups.[30,56] One study reported a significant increase in cell differentiation,[11] and 1 study reported a significant increase in interleukin 6 concentration.[65] A total of 7 studies reported increased collagen gene expression,[10,11,20,41,56,57,65] whereas 2 studies reported no difference.[30,56] Effects of PRP in vitro are reported in Table 2.

One study reported that PRP treatment significantly increased DNA content, metabolic cell activity, and production of matrix metalloproteinase (MMP)-3 and -13.[30] A separate study compared how 2 different PRP preparation systems, the Arthrex ACP Double Syringe System and Biomet GPS III, affected production and activity of MMP-2, -3, and -9. This study reported that GPS had higher total MMP-2, -3, and -9 concentrations for up to 144 hours whereas ACP had higher platelet-normalized MMP-2 and -3 concentrations.[51]

## In Vivo Ligament Publications

*PRP Composition And Reporting Trends.* The 31 in vivo ligament studies used various ligament models, including rat MCL,[2] canine ACL,[5,13,48,49,54,58,62,63] porcine ACL,[3,22,23,27,29,39,45-47] rabbit MCL,[14,26,31,40,66] rabbit ACL/CCL,[1,32,52,59,67] and human ACL (Appendix Table A3).[50,55,60] Among in vivo studies, detection and performance biases were consistently high, whereas selection, attrition, reporting, and other biases were consistently low (Table 3).

A total of 23 (74%) studies reported platelet concentrations in their final PRP preparation (Table 1).[**] All 23 reported platelet concentrations greater than those of whole blood. One study did not report the final platelet concentration, but the authors reported that there was a 2- to 3- fold increase from the peripheral blood count.[55]

One study reported leukocyte count but did not report its relative change,[52] 2 studies reported increased leukocyte counts,[31,58] and 2 studies reported decreased leukocyte counts.[1,32] One study reported an increased RBC count in the preparation,[31] whereas 1 study reported a relative decrease in RBC count.[32] No studies reported full cytology counts in their methods.

*Effects of PRP In Vivo.* A total of 5 studies examined cell proliferation and reported an increase in cell proliferation.[27,29,39,55,58] Further, 9 studies examined gene expression relevant to ligament healing[††] and found increased expression of COL1A1 (Collagen type I alpha 1 chain), COL3A1 (Collagen type III alpha 1 chain), TGF-β1(Transforming growth factor beta 1), biglycan, MMP-1 (Matrix metalloproteinase-1), MMP-13 (Matrix metalloproteinase-13), and TIMP-1 (Tissue inhibitor of metalloproteinases inhibitor 1), CD31 (Cluster of differentiation 31), VEGF (Vascular endothelial growth factor),

[#]References 10, 11, 36, 41, 56, 57, 64, 65.

[**]References 2, 3, 13, 14, 22, 23, 26, 27, 29, 31, 32, 39, 47, 50, 52, 54, 55, 59, 60, 62, 63, 66, 67.

[††]References 27, 29, 39, 55, 58, 59, 62, 63, 67.

TABLE 2
Summary of Variables Reported: In Vitro Studies

| Outcome | Studies Reporting, n (%) | Significant Increase, n | No Significant Change, n | Significant Decrease, n |
|---|---|---|---|---|
| Cell viability | 7 (58.3) | 5 | 2 | 0 |
| Cell proliferation | 2 (16.7) | 2 | 0 | 0 |
| Proteoglycan and collagen content | 5 (41.7) | 3 | 2 | 0 |
| Gene expression | 9 (75) | 7 | 2 | 0 |
| Cell migration | 1 (8.3) | 1 | 0 | 0 |
| Cell differentiation | 1 (8.3) | 1 | 0 | 0 |
| Inflammatory mediation | 1 (8.3) | 1 | 0 | 0 |

TSP-1 (Thrombospondin-1), NT-3 (Neurotrophin-3), GAP-43 (Growth associated protein-43), and NGF (Nerve growth factor), with the exception of 1 study, which found a decrease in TGF-β1 expression.[67] A total of 19 studies examined gross appearance of ligament repair, of which 4 studies[5,32,40,52] found a significant gross improvement in wound healing and ligament regeneration compared with controls and 15 studies found no difference when compared with controls[‡‡] (Table 4).

In total, 24 studies assessed ligament repair histologically, with 13 studies reporting significant improvements over control[§§] and 9 studies reporting no significant difference in improvements.[‖‖] One study reported a lower vascularity subscore, a lower ligament tissue maturity index score, and no inflammation with PRP treatment.[31] Other studies reported decreased inflammation in the PRP group compared with control groups.[1,14]

A total of 12 studies reported superior mechanical properties with PRP treatment, including maximum yield,[23,29,45,47,49] load to failure,[¶¶] linear stiffness,[3,22,29,45,67] tensile strength,[14] displacement at maximum load,[29] and work to maximum load.[3] One study was unable to assess mechanical properties because of inadequate control tissue properties.[40] In total, 3 studies found no difference in tensile load,[23,39,46] 4 studies found no difference in displacement,[23,39,46,58] 4 studies found no difference in laxity or flexion,[22,39,50,60] and 5 studies found no difference in stiffness.[39,46,49,58,59] One study found no difference in failure load, linear stiffness, tensile strength, laxity, displacement, and work to maximum load.[39] One study found that PRP treatment resulted in significantly less ligament strength, maximum load, displacement at maximum load, and work to maximum load compared with controls.[31] One study found significantly decreased laxity compared with controls.[45] An additional 3 studies[5,13,32] conducted a functional examination graded on established criteria and found improvements with PRP (Table 4).

One study analyzed changes in synovial fluid for degradative and inflammatory markers and found no significant differences.[5] As well, 3 studies checked for inflammatory markers: 2 studies found no significant difference,[50,54] and 1 study found a significant decrease.[60] One study reported that PRP increased fibrinogen content but decreased haptoglobin concentrations.[52]

## PRP Preparation Classification

Multiple concentrations of PRP were studied in some of the included investigations. Of the 43 included studies, only 1 study reported all necessary information to be classified by all schemes.[54] Additionally, no scheme was able to completely classify >30% of the studies included with the exception of the Ehrenfest system (53.4%). Of the included studies, 3 studies reported the dose of injected platelets in the PRP and were categorized as class A according to the Magalon system[31,45,54] 4 studies were categorized as class B[26,39,50,60]; 5 studies, as class C[1,5,13,17,52], and 13 studies, as class D.[##]

Only 1 study investigated the properties of leukocyte- and platelet-rich fibrin as defined by the Ehrenfest system,[48] and no study used pure platelet-rich fibrin. Only 2 studies reported an efficiency of production higher than class C via the Magalon system.[1,42] Of the 31 studies reporting the platelet count in the PRP, only 26 studies[a] reported the amount used. A summary of the number of studies that were successfully classified by the Magalon, Ehrenfest, PAW, and Sports Medicine schemes is provided in Table 5.

## DISCUSSION

The main findings of the current study were as follows. First, there existed considerable variability in cytology reporting and PRP protocols among all studies. Although the majority of individual studies reported positive findings with PRP treatment, the variability in PRP preparation methods and protocols limited the extent to which the data could support these findings and precluded the pooling of data. Second, the use of PRP for ligament injuries in the majority of in vivo and in vitro animal models conferred benefits at the cellular and molecular levels including increased collagen content, improved cell viability, and earlier regeneration times when compared with control groups. Third, PRP treatment in the majority of in vivo ligament injury models resulted in comparable gross

[‡‡]References 2, 13, 23, 26, 27, 29, 45–47, 55, 58, 59, 66, 67.
[§§]References 23, 27, 29, 39, 40, 47, 49, 52, 55, 58, 59, 66,67.
[‖‖]References 2, 5, 13, 22, 26, 32, 45, 48, 54.
[¶¶]References 3, 23, 27, 45, 47, 49, 59, 67.

[##] References 2, 14, 27, 29, 32, 36, 47, 51, 59, 62, 63, 66, 67.
[a]References 1, 2, 3, 10, 11, 13, 14, 22, 23, 26, 27, 29, 30, 31, 36, 39, 41, 50, 51, 52, 56, 57, 63, 64, 65, 66.

TABLE 3
SYRCLE[28] Bias Assessment of Included In Vivo Studies

| | SYRCLE Score (Low, High, or Unclear) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Lead Author (Year) | Selection Bias: Sequence Generation | Selection Bias: Baseline Characteristics | Selection Bias: Allocation Concealment | Performance Bias: Random Housing | Performance Bias: Blinding | Detection Bias: Random Outcome Assessment | Detection Bias: Blinding | Attrition Bias: Incomplete Outcome Data | Reporting Bias: Selective Outcome Reporting | Other: Other Sources of Bias |
| Agir (2017)[1] | Unclear | Unclear | High | High | Low | High | High | Low | High | Unclear |
| Amar (2015)[2] | Unclear | Low | Unclear | High | Low | High | High | Low | Low | Low |
| Biercevicz (2013)[3] | High | Low | High | High | Low | High | High | Low | Low | Low |
| Bozynski (2016)[5] | Unclear | Low | Unclear | Unclear | Low | High | High | Low | Low | Low |
| Cook (2016)[13] | Unclear | Low | Unclear | High | Low | High | High | Low | Low | Low |
| Costa (2017)[14] | Unclear | Low | Unclear | Low | Low | High | Low | Low | Low | Low |
| Fleming (2009)[23] | High | Low | High | High | Low | High | High | Low | Low | High |
| Fleming (2015)[22] | Unclear | Low | High | Unclear | Low | High | Low | Low | Low | Low |
| Harris (2012)[26] | Unclear | Low | Low | Unclear | Low | High | Low | Low | Low | Low |
| Haus (2012)[27] | High | Low | High | High | Low | High | High | Low | Low | Low |
| Joshi (2009)[29] | Unclear | Low | High | High | Low | High | Low | Low | Low | Low |
| LaPrade (2018)[31] | Unclear | Low | Low | Unclear | Low | High | Unclear | Low | Low | Low |
| Lee (2012)[32] | Unclear | Low | High | High | Low | High | High | Low | Low | Unclear |
| Mastrangelo (2011)[39] | Low | Low | Low | Unclear | Low | High | Low | Low | Low | Low |
| Matsunaga (2013)[40] | High | Low | High | High | Low | High | High | Low | Low | Unclear |
| Murray (2006)[49] | Unclear | Low | High | High | Low | High | High | Low | Low | Low |
| Murray (2007)[48] | High | Low | High | High | Low | High | Low | Low | Low | Low |
| Murray (2007)[47] | Unclear | Low | High | High | Low | High | High | Low | Low | Low |
| Murray (2009)[46] | Low | Low | High | High | Low | High | High | Low | Low | Low |
| Murray (2013)[45] | Low | Low | Low | High | Low | High | Low | Low | Low | Low |
| Prządka (2017)[52] | High | Low | Low | Unclear | Low | High | Low | Low | Low | Low |
| Sample (2018)[54] | High | Unclear | High | High | Low | High | High | Unclear | Low | Unclear |
| Smith (2020)[58] | Unclear | Low | Low | High | Low | High | Low | Low | Low | Low |
| Teng (2016)[59] | Unclear | Low | High | High | Low | High | High | Low | Low | Low |
| Xie (2013)[62] | Unclear | Low | Unclear | High | Low | High | High | Low | Low | Low |
| Xie (2013)[63] | Unclear | Low | Unclear | High | Low | High | High | Low | Low | Low |
| Yoshioka (2013)[66] | High | Low | Low | Unclear | Low | High | Low | Low | Low | Low |
| Zhang (2019)[67] | Unclear | Low | Unclear | High | Low | High | Low | Low | Low | Low |

appearances but superior biomechanical performance when compared with controls.

The PRP preparation methods were significantly heterogeneous among the identified studies. Although the majority of basic science evidence that evaluated the use of PRP for the treatment of ligament pathologies in the current review reported improvements in various aspects of ligament healing, it remains a challenge to appropriately compare these changes across studies. Despite increasing calls for standardization in methods and reporting, rigorous and reproducible standards have not been adopted. In the current study, each included study was classified by 4 established classification schemes when applicable: Magalon,[35] Erhenfest,[18] PAW,[15] and Sports Medicine.[43] Only 1 study reported all requisite information to be classified by all schemes.[54] Additionally, only the Ehrenfest system was able to classify >30% of studies. This was largely because of the fact that most studies did not report information on leukocyte concentrations, specifically regarding changes in neutrophil concentrations. Inflammatory neutrophil content has been cited to directly alter platelet function and play an important role in stimulating the healing process.[6,25] Future basic science studies may be at risk of contributing little to the

TABLE 4
Summary of Variables Reported: In Vivo Studies

| Outcome | Studies Reporting, n (%) | Significant Increase, n | No Significant Change, n | Significant Decrease, n |
|---|---|---|---|---|
| Cell viability | 0 (0) | 0 | 0 | 0 |
| Cell proliferation | 5 (16.1) | 5 | 0 | 0 |
| Gene expression | 9 (29.0) | 8 | 0 | 1 |
| Gross appearance of repair | 18 (58.1) | 3 | 15 | 0 |
| Histology of repair | 24 (77.4) | 13 | 9 | 2 |
| Collagen deposition | 7 (22.6) | 6 | 0 | 1 |
| Mechanical properties | 23 (74.2) | 12 | 9 | 2 |
| Inflammatory mediation | 6 (19.4) | 0 | 4 | 2 |

TABLE 5
Summary of Platelet-Rich Plasma Classifications Systems[a]

| Classification | Yes, n (%) | No, n (%) |
|---|---|---|
| Magalon (D): dose of injected platelets | 25 (58.1) | 18 (41.9) |
| Magalon (E): efficiency of production | 17 (39.5) | 26 (60.5) |
| Magalon (P): purity of the PRP | 8 (18.6) | 35 (81.4) |
| Magalon (A): activation of the PRP | 43 (100.0) | 0 (0.0) |
| Ehrenfest: P-PRP, L-PRP, P-PRF, L-PRF | 23 (53.4) | 20 (46.6) |
| PAW (P): platelet count | 31 (72.1) | 12 (27.9) |
| PAW (A): activation method | 43 (100.0) | 0 (0.0) |
| PAW (W): white blood count | 12 (27.9) | 31 (72.1) |
| Sports Medicine (Mishra) classification | 9 (20.9) | 34 (79.1) |

[a]L-PRF, leukocyte- and platelet-rich fibrin; L-PRP, leukocyte- and platelet-rich plasma; P-PRF, pure platelet-rich fibrin; P-PRP, pure platelet-rich plasma; PRP, platelet-rich plasma.

current level of evidence and understanding of PRP indications and efficacy unless there is widespread adoption of formal recommendations and classification systems that include quantitative characteristics of PRP processing. Adoption of clear and consistent reporting by authors and journals is essential to the development of well-defined clinical indications for PRP therapy and cellular composition targets to guide further optimization of patient selection criteria and harvest and processing strategies.

Several cellular and molecular events thought to lead to more efficient ligament healing, including cell differentiation, proliferation, and collagen expression, were found to be more prominent in PRP-treated ligament models compared with controls in select studies. However, meaningful conclusions could not be drawn because of study heterogeneity and the fact that some studies presented conflicting data. Studies on the effects of PRP on ligament pathology suggested that PRP treatment is beneficial for overall ligament healing via a variety of cellular and molecular mechanisms.[23,45,48,66] However, it should be noted that this finding was derived from data from 60% of in vitro and 25% of in vivo studies. Type I and type III collagen are integral components of ligaments, providing tensile strength and a fibrillar component to remodeling, respectively.[62] In both in vitro and in vivo studies, collagen content was observed to significantly increase with PRP treatment.[11,20,65] Matsunaga et al[40] reported that their compact PRP-rich scaffold enhanced ACL healing in a rabbit model by generating denser and more longitudinal collagen bundles that were better organized than those of controls. Collagen production was also measured at the level of gene expression. Although Fallouh et al[20] found significant increases in only COL3A1 expression, numerous studies reported increases in both COL1A1 and COL3A1 expression.[11,62,64,65] Further research is necessary to clarify whether the increased collagen synthesis induced by PRP in ligaments is translated to improved clinical and functional outcomes.

Various cellular changes were also observed in ligament models treated with PRP when compared with controls. Increases in cell viability,[10,11,20,64,65] cellular differentiation,[20] and proinflammatory markers[65] were reported with PRP treatment and may be beneficial to ligament healing. Furthermore, increases in an array of genes implicated in promoting extracellular matrix synthesis and the remodeling aspect of healing (TGF-β1, biglycan, MMP-1, MMP-13, TIMP-1), angiogenesis (CD31, VEGF, TSP-1), and markers of neurotrophic support and axonal regeneration (NT-3, GAP-43, NGF) were observed in multiple studies,[62,63] supporting a diverse set of mechanisms by which PRP may promote efficacious ligament healing. At the histological level, PRP significantly increased cellularity and regeneration,[23,40,52,66] increased angiogenesis,[23] and promoted earlier and more organized ligament filling.[48,49,52] These changes suggest that PRP treatment induces a variety of cellular changes that may expedite and enhance ligament regeneration and healing; however, results from a few other studies contradicted these findings. Indeed, 2 in vitro ligament studies found no significant change in cell viability,[11,17] 2 studies found no change in collagen content,[30,56] and 2 studies found no change in gene expression.[30,56] One study found a decrease in TGF-β1 expression.[67] Therefore, although the majority of studies reported benefits with PRP, there is also a body of evidence suggesting that these observations are not consistent.

A minority of studies found qualitative macroscopic improvements in tissue repair and appearance after treatment with PRP that were significant when compared with controls,[5,32,40,52] although the majority of studies investigating this gross ligament appearance did not. Interestingly, biomechanical performance including stiffness, yield, and maximum and failure loads were found to be superior in PRP-treated groups in comparison to control

groups.[22,23,40,47,49,66] This suggests that PRP-induced changes at the cellular level may translate into enhanced biomechanical performance of ligaments although the resultant gross appearance of ligaments may not reflect these benefits. It would be speculative to suggest that PRP has the potential to enhance the ligament healing process based on these data. Regardless, the current review suggests that the treatment of ligament injury models with PRP in a laboratory environment confers many diverse benefits, when compared with control models, via numerous cellular and molecular mediators. These changes may have implications for clinical treatment; however, there remains a need to better understand how the magnitude and timing of these changes occur with different PRP protocols, compositions, and formulations.

This systematic review is subject to limitations inherent in all systematic reviews, namely by the studies identified for analysis. First, a large number of ligament model studies did not report final platelet concentrations in their PRP preparations.[b] Although this prevented a systematic comparison of final PRP preparations across all studies, it did not limit a comparison of the cellular and molecular markers that changed with the use of PRP in comparison to controls, as was our primary endpoint. Second, this systematic review analyzed a heterogeneous selection of studies that included both in vitro and in vivo investigations, and this heterogeneity precluded quantitative comparisons among articles with similar study design (ie, among in vitro studies only). We believe that this heterogeneity also speaks to the need for standardization among protocols, as they differed in most studies. We also believe that this heterogeneity was in fact a benefit to the study in that it allowed for extraction and documentation of many different cellular and molecular markers in PRP treatments that may not have otherwise been noted. Third, in many of the studies examining ligament injuries, these injuries were surgically induced. This limitation was apparent in that PRP use was applied clinically to injuries that were secondary to trauma or overuse. We cannot comment on how results may differ following the use of PRP for surgically induced injuries versus trauma-related or chronically induced ligament injuries. Future studies should aim to investigate these differences, if any, and potentially investigate the use of PRP in chronic or traumatic injuries to more intimately mirror natural ligament injuries.

## CONCLUSION

Conflicting data on the cellular and molecular effects of PRP for ligament injuries were observed secondary to the finding that included studies were heterogeneous, limiting interpretation across studies and the ability to draw meaningful conclusions. Clinical trials and any causal relationship between PRP use in ligament injuries and its potential for regeneration and healing should be pursued with caution if based solely on basic science data. The lack of reporting and consistency in PRP preparation methods is concerning, in agreement with previous literature urging standardization, and must be addressed prior to investment of significant resources in human clinical trials.

[b]References 5, 13, 17, 20, 32, 40, 46, 48, 49, 59.

## REFERENCES


1. Agir I, Aytekin MN, Kucukdurmaz F, Kocaoglu B, Cetinel S, Karahan M. The effect of platelet-rich plasma in bone-tendon integration. *Adv Clin Exp Med*. 2017;26(2):193-199.
2. Amar E, Snir N, Sher O, et al. Platelet-rich plasma did not improve early healing of medial collateral ligament in rats. *Arch Orthop Trauma Surg*. 2015;135(11):1571-1577.
3. Biercevicz AM, Miranda DL, Machan JT, Murray MM, Fleming BC. In situ, noninvasive, T2*-weighted MRI-derived parameters predict ex vivo structural properties of an anterior cruciate ligament reconstruction or bioenhanced primary repair in a porcine model. *Am J Sports Med*. 2013;41(3):560-566.
4. Boswell SG, Cole BJ, Sundman EA, Karas V, Fortier LA. Platelet-rich plasma: a milieu of bioactive factors. *Arthroscopy*. 2012;28(3):429-439.
5. Bozynski CC, Stannard JP, Smith P, et al. Acute management of anterior cruciate ligament injuries using novel canine models. *J Knee Surg*. 2016;29(7):594-603.
6. Caruana A, Savina D, Macedo JP, Soares SC. From platelet-rich plasma to advanced platelet-rich fibrin: biological achievements and clinical advances in modern surgery. *Eur J Dent*. 2019;13(2):280-286.
7. Chahla J, Cinque ME, Piuzzi NS, et al. A call for standardization in platelet-rich plasma preparation protocols and composition reporting: a systematic review of the clinical orthopaedic literature. *J Bone Joint Surg Am*. 2017;99(20):1769-1779.
8. Chen X, Jones IA, Park C, Vangsness CT Jr. The efficacy of platelet-rich plasma on tendon and ligament healing: a systematic review and meta-analysis with bias assessment. *Am J Sports Med*. 2018;46(8):2020-2032.
9. Chen X, Jones IA, Togashi R, Park C, Vangsness CT Jr. Use of platelet-rich plasma for the improvement of pain and function in rotator cuff tears: a systematic review and meta-analysis with bias assessment. *Am J Sports Med*. 2020;48(8):2028-2041.
10. Cheng M, Johnson VM, Murray MM. Effects of age and platelet-rich plasma on ACL cell viability and collagen gene expression. *J Orthop Res*. 2012;30(1):79-85.
11. Cheng M, Wang H, Yoshida R, Murray MM. Platelets and plasma proteins are both required to stimulate collagen gene expression by anterior cruciate ligament cells in three-dimensional culture. *Tissue Eng Part A*. 2010;16(5):1479-1489.
12. Clayton RA, Court-Brown CM. The epidemiology of musculoskeletal tendinous and ligamentous injuries. *Injury*. 2008;39(12):1338-1344.
13. Cook JL, Smith PA, Bozynski CC, et al. Multiple injections of leukoreduced platelet rich plasma reduce pain and functional impairment in a canine model of ACL and meniscal deficiency. *J Orthop Res*. 2016;34(4):607-615.
14. Costa ELD, Teixeira LEM, Padua BJ, Araujo ID, Vasconcellos LS, Dias LSB. Biomechanical study of the effect of platelet rich plasma on the treatment of medial collateral ligament lesion in rabbits. *Acta Cir Bras*. 2017;32(10):827-835.
15. DeLong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. *Arthroscopy*. 2012;28(7):998-1009.
16. Del Torto M, Enea D, Panfoli N, Filardo G, Pace N, Chiusaroli M. Hamstrings anterior cruciate ligament reconstruction with and without platelet rich fibrin matrix. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(12):3614-3622.
17. Dhillon MS, Karna SK, Dhatt SS, Behera P, Bhatia A. Can platelet rich plasma stimulate human ACL growth in culture? A preliminary experience. *Muscles Ligaments Tendons J*. 2015;5(3):156-161.
18. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (platelet-rich plasma-PRP, platelet-rich fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. *Muscles Ligaments Tendons J*. 2014;4(1):3-9.

19. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009;27(3):158-167.
20. Fallouh L, Nakagawa K, Sasho T, et al. Effects of autologous platelet-rich plasma on cell viability and collagen synthesis in injured human anterior cruciate ligament. *J Bone Joint Surg Am*. 2010;92(18): 2909-2916.
21. Filardo G, Di Matteo B, Kon E, Merli G, Marcacci M. Platelet-rich plasma in tendon-related disorders: results and indications. *Knee Surg Sports Traumatol Arthrosc*. 2018;26(7):1984-1999.
22. Fleming BC, Proffen BL, Vavken P, Shalvoy MR, Machan JT, Murray MM. Increased platelet concentration does not improve functional graft healing in bio-enhanced ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(4):1161-1170.
23. Fleming BC, Spindler KP, Palmer MP, Magarian EM, Murray MM. Collagen-platelet composites improve the biomechanical properties of healing anterior cruciate ligament grafts in a porcine model. *Am J Sports Med*. 2009;37(8):1554-1563.
24. Garcia FL, Williams BT, Polce EM, et al. Preparation methods and clinical outcomes of platelet-rich plasma for intra-articular hip disorders: a systematic review and meta-analysis of randomized clinical trials. *Orthop J Sports Med*. 2020;8(10):2325967120960414.
25. Goel MS, Diamond SL. Neutrophil cathepsin G promotes prothrombinase and fibrin formation under flow conditions by activating fibrinogen-adherent platelets. *J Biol Chem*. 2003;278(11):9458-9463.
26. Harris NL, Huffer WE, von Stade E, Larson AI, Phinney S, Purnell ML. The effect of platelet-rich plasma on normal soft tissues in the rabbit. *J Bone Joint Surg Am*. 2012;94(9):786-793.
27. Haus BM, Mastrangelo AN, Murray MM. Effect of anterior cruciate healing on the uninjured ligament insertion site. *J Orthop Res*. 2012;30(1):86-94.
28. Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14(1):43.
29. Joshi SM, Mastrangelo AN, Magarian EM, Fleming BC, Murray MM. Collagen-platelet composite enhances biomechanical and histologic healing of the porcine anterior cruciate ligament. *Am J Sports Med*. 2009;37(12):2401-2410.
30. Krismer AM, Cabra RS, May RD, et al. Biologic response of human anterior cruciate ligamentocytes on collagen-patches to platelet-rich plasma formulations with and without leucocytes. *J Orthop Res*. 2017;35(12):2733-2739.
31. LaPrade RF, Goodrich LR, Phillips J, et al. Use of platelet-rich plasma immediately after an injury did not improve ligament healing, and increasing platelet concentrations was detrimental in an in vivo animal model. *Am J Sports Med*. 2018;46(3):702-712.
32. Lee AJ, Chung WH, Kim DH, et al. Anterior cruciate ligament reconstruction in a rabbit model using canine small intestinal submucosa and autologous platelet-rich plasma. *J Surg Res*. 2012;178(1):206-215.
33. Lin MT, Chiang CF, Wu CH, Huang YT, Tu YK, Wang TG. Comparative effectiveness of injection therapies in rotator cuff tendinopathy: a systematic review, pairwise and network meta-analysis of randomized controlled trials. *Arch Phys Med Rehabil*. 2019;100(2):336-349.
34. 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(2):269-278.
35. Magalon J, Chateau AL, Bertrand B, et al. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. *BMJ Open Sport Exerc Med*. 2016;2(1):e000060.
36. Magarian EM, Vavken P, Murray MM. Human anterior cruciate ligament fibroblasts from immature patients have a stronger in vitro response to platelet concentrates than those from mature individuals. *Knee*. 2011;18(4):247-251.
37. Magnussen RA, Flanigan DC, Pedroza AD, Heinlein KA, Kaeding CC. Platelet rich plasma use in allograft ACL reconstructions: two-year clinical results of a MOON cohort study. *Knee*. 2013;20(4):277-280.
38. Marx RE. Platelet-rich plasma: evidence to support its use. *J Oral Maxillofac Surg*. 2004;62(4):489-496.
39. Mastrangelo AN, Vavken P, Fleming BC, Harrison SL, Murray MM. Reduced platelet concentration does not harm PRP effectiveness for ACL repair in a porcine in vivo model. *J Orthop Res*. 2011;29(7): 1002-1007.
40. Matsunaga D, Akizuki S, Takizawa T, Omae S, Kato H. Compact platelet-rich fibrin scaffold to improve healing of patellar tendon defects and for medial collateral ligament reconstruction. *Knee*. 2013;20(6):545-550.
41. McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. *J Orthop Res*. 2009;27(8):1033-1042.
42. Mirzatolooei F, Alamdari MT, Khalkhali HR. The impact of platelet-rich plasma on the prevention of tunnel widening in anterior cruciate ligament reconstruction using quadrupled autologous hamstring tendon: a randomised clinical trial. *Bone Joint J*. 2013;95(1):65-69.
43. Mishra A, Harmon K, Woodall J, Vieira A. Sports medicine applications of platelet rich plasma. *Curr Pharm Biotechnol*. 2012;13(7): 1185-1195.
44. Murray IR, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum information for studies evaluating biologics in orthopaedics (MIBO): platelet-rich plasma and mesenchymal stem cells. *J Bone Joint Surg Am*. 2017;99(10):809-819.
45. Murray MM, Fleming BC. Use of a bioactive scaffold to stimulate anterior cruciate ligament healing also minimizes posttraumatic osteoarthritis after surgery. *Am J Sports Med*. 2013;41(8):1762-1770.
46. Murray MM, Palmer M, Abreu E, Spindler KP, Zurakowski D, Fleming BC. Platelet-rich plasma alone is not sufficient to enhance suture repair of the ACL in skeletally immature animals: an in vivo study. *J Orthop Res*. 2009;27(5):639-645.
47. Murray MM, Spindler KP, Abreu E, et al. Collagen-platelet rich plasma hydrogel enhances primary repair of the porcine anterior cruciate ligament. *J Orthop Res*. 2007;25(1):81-91.
48. Murray MM, Spindler KP, Ballard P, Welch TP, Zurakowski D, Nanney LB. Enhanced histologic repair in a central wound in the anterior cruciate ligament with a collagen-platelet-rich plasma scaffold. *J Orthop Res*. 2007;25(8):1007-1017.
49. Murray MM, Spindler KP, Devin C, et al. Use of a collagen-platelet rich plasma scaffold to stimulate healing of a central defect in the canine ACL. *J Orthop Res*. 2006;24(4):820-830.
50. 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(11):1206-1213.
51. Pifer MA, Maerz T, Baker KC, Anderson K. Matrix metalloproteinase content and activity in low-platelet, low-leukocyte and high-platelet, high-leukocyte platelet rich plasma (PRP) and the biologic response to PRP by human ligament fibroblasts. *Am J Sports Med*. 2014;42(5): 1211-1218.
52. Prządka P, Kielbowicz Z, Osinski B, et al. Reconstruction of cranial cruciate ligament in rabbits using polyester implants saturated with PRP, antlerogenic stem cells MIC-1 and their homogenate. *Connect Tissue Res*. 2017;58(5):464-478.
53. Rodeo SA, Delos D, Weber A, et al. What's new in orthopaedic research. *J Bone Joint Surg Am*. 2010;92(14):2491-2501.
54. Sample SJ, Racette MA, Hans EC, et al. Use of a platelet-rich plasma-collagen scaffold as a bioenhanced repair treatment for management of partial cruciate ligament rupture in dogs. *PLoS One*. 2018;13(6): e0197204.
55. Sánchez M, Anitua E, Azofra J, Prado R, Muruzabal F, Andia I. Ligamentization of tendon grafts treated with an endogenous preparation rich in growth factors: gross morphology and histology. *Arthroscopy*. 2010;26(4):470-480.
56. Schnabel LV, Sonea HO, Jacobson MS, Fortier LA. Effects of platelet rich plasma and acellular bone marrow on gene expression patterns and DNA content of equine suspensory ligament explant cultures. *Equine Vet J*. 2008;40(3):260-265.
57. Smith JJ, Ross MW, Smith RK. Anabolic effects of acellular bone marrow, platelet rich plasma, and serum on equine suspensory ligament fibroblasts in vitro. *Vet Comp Orthop Traumatol*. 2006;19(1):43-47.
58. Smith PA, Stannard JP, Bozynski CC, Kuroki K, Cook CR, Cook JL. Patellar bone-tendon-bone autografts versus quadriceps tendon

allograft with synthetic augmentation in a canine model. *J Knee Surg*. 2020;33(12):1256-1266.
59. Teng C, Zhou C, Xu D, Bi F. Combination of platelet-rich plasma and bone marrow mesenchymal stem cells enhances tendon-bone healing in a rabbit model of anterior cruciate ligament reconstruction. *J Orthop Surg Res*. 2016;11(1):96.
60. Valenti Azcarate A, Lamo-Espinosa J, Aquerreta Beola JD, Hernandez Gonzalez M, Mora Gasque G, Valenti Nin JR. Comparison between two different platelet-rich plasma preparations and control applied during anterior cruciate ligament reconstruction: is there any evidence to support their use? *Injury*. 2014;45(suppl 4):S36-S41.
61. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg*. 1997;55(11):1294-1299.
62. Xie X, Wu H, Zhao S, Xie G, Huangfu X, Zhao J. The effect of platelet-rich plasma on patterns of gene expression in a dog model of anterior cruciate ligament reconstruction. *J Surg Res*. 2013;180(1):80-88.
63. Xie X, Zhao S, Wu H, et al. Platelet-rich plasma enhances autograft revascularization and reinnervation in a dog model of anterior cruciate ligament reconstruction. *J Surg Res*. 2013;183(1):214-222.
64. Yoshida R, Cheng M, Murray MM. Increasing platelet concentration in platelet-rich plasma inhibits anterior cruciate ligament cell function in three-dimensional culture. *J Orthop Res*. 2014;32(2):291-295.
65. Yoshida R, Murray MM. Peripheral blood mononuclear cells enhance the anabolic effects of platelet-rich plasma on anterior cruciate ligament fibroblasts. *J Orthop Res*. 2013;31(1):29-34.
66. Yoshioka T, Kanamori A, Washio T, et al. The effects of plasma rich in growth factors (PRGF-Endoret) on healing of medial collateral ligament of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2013;21(8):1763-1769.
67. Zhang M, Zhen J, Zhang X, et al. Effect of autologous platelet-rich plasma and gelatin sponge for tendon-to-bone healing after rabbit anterior cruciate ligament reconstruction. *Arthroscopy*. 2019;35(5):1486-1497.

## APPENDIX

TABLE A1
Reporting Trends for Biologic Changes Induced by PRP[a]

| | Studies Reporting, Total (In Vivo/In Vitro), n | Increased, Decreased, or No Change (In Vivo/In Vitro) |
|---|---|---|
| Growth factor concentrations | | |
| EGF | 1 (1/0) | Increased, not reported |
| PDGF A + B | 4 (3/1) | Increased, decreased |
| TGF-β1 | 5 (4/1) | 3 increased, 1 decreased/1 decreased |
| IGF-1, IGF-2 | 1 (1/0) | No change, not reported |
| VEGF, ECGF | 6 (6/0) | Increased, not reported |
| bFGF | 0 | Not reported |
| FGF-2 | 1 (1/0) | Increased, not reported |
| FGF-18 | 0 | Not reported |
| BMP-2 | 0 | Not reported |
| BMP-7 | 0 | Not reported |
| HGF | 0 | Not reported |
| Adhesive protein concentration | | |
| Fibrinogen | 4 (3/1) | 2 increased, 1 no change/increased |
| Fibronectin | 1 (1/0) | Increased, not reported |
| Vitronectin | 0 | Not reported |
| Thrombospondin-1 | 1 (0/1) | Not reported, increased |
| Clotting factor concentration | | |
| Factor V | 0 | Not reported |
| Factor XI | 0 | Not reported |
| Protein S | 0 | Not reported |
| Anti-thrombin | 0 | Not reported |
| Fibrinolytic factors | | |
| Plasminogen | 0 | Not reported |
| Plasminogen activator inhibitor | 0 | Not reported |
| $\alpha_2$ antiplasmin | 0 | Not reported |
| Proteases and antiproteases | | |
| TIMP-4 | 0 | Not reported |
| Metalloprotease-4 | 0 | Not reported |
| $\alpha_1$-antitrypsin | 0 | Not reported |
| Basic proteins | | |
| Platelet factor 4 | 0 | Not reported |
| B-thromboglobulin | 0 | Not reported |
| Endostatins | 0 | Not reported |
| Membrane glycoproteins | | |
| CD40 ligand | 0 | Not reported |
| P-selectin | 0 | Not reported |
| Dense granule bioactive molecules | | |
| Serotonin | 0 | Not reported |
| Histamine | 0 | Not reported |
| Dopamine | 0 | Not reported |
| ADP | 0 | Not reported |
| ATP | 0 | Not reported |
| $Ca^{2+}$ | 0 | Not reported |
| Catecholamines | 0 | Not reported |
| Proinflammatory cytokine concentration | | |
| IL-α | 0 | Not reported |
| IL-1β | 0 | Not reported |
| IL-2 | 0 | Not reported |
| IL-6 | 1 (0/1) | Not reported, increased |
| IL-7 | 0 | Not reported |
| IL-8 (CXCL8) | 1 (1/0) | No change |
| TNF-α | 0 | Not reported |
| IFN-α | 0 | Not reported |
| IL-12 | 0 | Not reported |
| IL-15 | 0 | Not reported |
| IL-17 | 0 | Not reported |
| IL-18 | 0 | Not reported |
| NK-B | 0 | Not reported |
| Anti-inflammatory cytokine concentration | | |
| IL-1 receptor antagonist | 0 | Not reported |
| IL-4 | 0 | Not reported |
| IL-5 | 0 | Not reported |
| IL-10 | 0 | Not reported |
| IL-13 | 0 | Not reported |
| IFN-γ | 0 | Not reported |
| Other proteins | | |
| Activin A | 0 | Not reported |

Table A1 (continued)

| | Studies Reporting, Total (In Vivo/In Vitro), n | Increased, Decreased, or No Change (In Vivo/In Vitro) |
|---|---|---|
| AGE | 0 | Not reported |
| Agrin | 0 | Not reported |
| BDNF | 0 | Not reported |
| CCL2 | 0 | Not reported |
| CCL5 | 0 | Not reported |
| CCL20 | 0 | Not reported |
| CXCL1 | 0 | Not reported |
| CXCL2 | 0 | Not reported |
| CXCL3 | 0 | Not reported |
| CXCL5 | 0 | Not reported |
| CXCL7 | 0 | Not reported |
| CXCL10 | 0 | Not reported |
| CNTF | 0 | Not reported |
| CD86 | 0 | Not reported |
| CSF2 | 0 | Not reported |
| Fas ligand | 0 | Not reported |
| Fractalkine | 0 | Not reported |
| ICAM1 | 0 | Not reported |
| IL-1 receptor-like 2 | 0 | Not reported |
| L-selectin | 0 | Not reported |
| Leptin | 0 | Not reported |
| MMP-1 | 2 (2/0) | 1 increased, 1 no change/not reported |
| MMP-2 | 2 (1/1) | No change/1 increased |
| MMP-3 | 5 (1/4) | No change/2 increased, 1 decreased, 1 no change |
| MMP-8 | 0 | Not reported |
| MMP-9 | 1 (0/1) | Not reported/1 increased |
| MMP-13 | 4 (1/3) | Increased/1 increased, 1 decreased, 1 no change |
| Prolactin receptor | 0 | Not reported |
| TIMP-1 | 1 (1/0) | Increased, not reported |
| RANTES | 0 | Not reported |
| MCP-1 | 1 (1/0) | No change, not reported |
| MIP-1a | 0 | Not reported |
| G-CSF | 0 | Not reported |

[a]ADP, adenosine diphosphate; AGE, advanced glycosylation end product; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CNTF, ciliary neurotrophic factor; CSF, colony-stimulating factor; CXCL, chemokine (C-X-C motif) ligand; ECGF, endothelial cell growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factors; G-CSF, granulocyte colony stimulating factor; HGF, hepatocyte growth factor; ICAM, intercellular adhesion molecule; IFN, interferon; IGF, insulin-like growth factor; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage Inflammatory Protein; MMP, matrix metalloproteinase; NK, natural killer; PDGF, platelet-derived growth factor; RANTES, regulated on Activation Normal T cell Expressed and Secreted; TGF, tumor growth factor; TNF, tumor necrosis factor; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

TABLE A2
Summary of Included In Vitro Ligament Studies[a]

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Cheng (2010)[11] | 20 samples of blood (10 mL/tube) centrifuged at 150*g* for 6 min. Supernatant combined and transferred to 50-mL tube as PRP. | Platelet count: 801 $\times 10^6$/mL | ACL explants cultured in control media, PRP, PPP, or platelets alone. | Viability assessed via metabolism MTT and TUNEL assays for apoptosis. Histology/TEM for cell morphology. IMHC for collagen deposition. RT-PCR GAPDH, COL1A1, and COL3A1. | PRP did not differ from PPP and platelets in viability. Histologically, PRP had significantly better results and expressed higher collagen levels. |
| Cheng (2012)[10] | Porcine blood (300 mL) collected with 10% acid-citrate dextrose, centrifuged at 150*g*/6 min. Supernatant combined to yield PRP. | Platelet count: 628 $\times 10^6$/mL | ACL explants removed from 3 different aged donor pigs (immature, adolescent, and adult) cultured in collagen hydrogel PRP. | RT-PCR for COL1A1 and COL3A1. TUNEL assay for apoptosis. MTT for metabolic level (viability) and PicoGreen dsDNA for DNA content. | PRP increased viability, decreased apoptosis, and increased the expression of collagen. Results were more favorable for immature and adolescent cells vs adult cells. |

Table A2 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Dhillon (2015)[17] | Antecubital vein isolated and transferred to tubes. Tubes centrifuged; PRP isolated via a pipette in first tube. Second tube centrifuged at 3000 rpm; PPP separated. | Not provided | ACL explants recovered from arthroscopic reconstruction and subsequently cultured in either in 5% or 10% PRP. | MTT assay, annexin V assay (apoptosis), and DNA content/S-phase fraction. | No significant difference was noted between the control media and that containing PRP. |
| Fallouh (2010)[20] | Platelet concentration system (DePuy Symphony) used to isolate PRP and PPP from blood. Both isolated plasmas treated with 10% thrombin solution (100 U/mL) and centrifuged at 1500*g* for 5 min. Soluble releasates frozen at –80°C. | Not provided | ACL explants cultured in PRP. | Viability (WST-8 assay), Sircol assay (collagen content), and RT-PCR for COL1A1 and COL3A1. | PRP increased viability, collagen content, and the expression of 3 genes. There was no increase in type 1 gene expression. |
| Krismer (2017)[30] | 60-mL blood samples from human male donors added to citrate dextrose. Blood samples centrifuged and separated by weight via (1) Platelets Matter/GPS III commercial kit (PRP-LR) and (2) Yoshida et al[65] protocol (PRP-PP). Activated via 1.5 U/mL thrombin. Cell composition determined using ADIVA 2120i System.[c] | • PRP-LR platelet count: 813.8 ± 233.7 × $10^9$/L<br>• PRP-LR WBC count: 15.91 ± 8.32 × $10^9$/L<br>• PRP-PP RBC count: 1.27 ± 1.31 × $10^{12}$/L<br>• PRP-PP platelet count: 844.1 ± 126.1 × $10^9$/L<br>• WBC count: 0.43 ± 0.38 × $10^9$/L<br>• RBC count: 0.08 ± 0.03 × $10^{12}$/L | 3 experimental groups: 2.5% PRP-LR, 2.5% PRP-PP, 20% PRP-LR; plus a negative control and a positive control. | Cell proliferation, cell phenotype on mRNA transcript level, and extracellular matrix production (total collagen and GAG content). | PRP significantly increased DNA content and metabolic cell activity at day 21 vs day 7. PRP significantly increased cell proliferation but not extracellular matrix production. PRP-LR group significantly increased MMP-3 and MMP-13 expression; no significant increases in COL1A2, scleraxin A, aggrecan, or tenomodulin across all groups. |
| Magarian (2011)[36] | 600 mL whole human blood collected with acid-citrate dextrose (10% by volume) and centrifuged at 150*g* for 6 min. Supernatant called PRP. PRP centrifuged, and PPP removed. Pellet resuspended and concentrated with PPP to approximately 3× whole blood. | • Platelet count: 684 × $10^9$/L<br>• Whole-blood platelet count: 202 × $10^9$/L<br>• PPP platelet count: 154 × $10^9$/L | ACL explants harvested from immature and adolescent children were cultured in PRP. Investigators evaluated the age differences in response in migration, viability, and scaffold contracture. | Proliferation (MTT assay), migration (Boyden chamber), and scaffold contracture. | Immature ACL explant cells treated with PRP had increased migration at 24 h and proliferation at 7 and 14 d vs those of adolescents. |
| McCarrel (2009)[41] | Bone marrow biopsy needle used to obtain sternal bone marrow, which was combined with heparin (33 U/mL). Venous blood (60 mL) centrifuged at 400*g* for 20 min to yield PRP (10 mL). | • Whole-blood count: 100 × $10^3$ platelets/μL<br>• Plasma: 82.6 × $10^3$ platelets/μL<br>• Platelet count: 513 ± 84.9 × 1000/μL | Suspensory ligament cultured in BMA, PRP, or lyophilized platelet product. | RT-PCR for COL1A1, COL3A1, COMP, decorin, MMP-3, MMP-13. | PRP increased expression of COL1A1, COMP, and decorin. PRP decreased expression of COL3A1, MMP-3, and MMP-13. |

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Table A2 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Pifer (2014)[51] | • Releasing kinetics of MMP-2, -3, -9 from PRP: venous blood was obtained from 2 healthy donors. PRP was made using ACP with 1 donor and GPS with the other donor. Each PRP was split into three 500-μL parts and exposed to 500-μL of release medium, 1% FBS, and 1% penicillin/streptomycin. Samples were incubated at 37°C for 30 min and then withdrawn at 0, 24, 48, 96, 120, and 144 h after the initial 30-min period. Samples were centrifuged at 1500 rpm for 10 min. Total MMP-2, -3, and -9 concentrations were assayed using multiplex ELISA.<br>• Endogenous and total active MMP-2, -3, and -9 in PRP: venous blood was obtained from 3 healthy donors from the blood bank. PRP was processed in the same manner as the previous methods, except samples were incubated for 24 h at 37°C without being disturbed. The samples were then used to quantify endogenously active and total MMP. | • Releasing kinetics: whole-blood platelet count = 317 and 217 × $10^3$/μL for donors 1 and 2, respectively<br>• ACP platelet count: 418 × $10^3$/μL<br>• GPS platelet count: 1268 × $10^3$/μL<br>• Total activity: whole-blood platelet count: 219.5 ± 82.5 × $10^3$/μL<br>• ACP platelet count: 386.4 ± 163.9 × $10^3$/μL<br>• GPS platelet count: 1131.5 ± 497.3 × $10^3$/μL | 3 groups of human periodontal ligament fibroblasts were cultured and exposed to both PRP-ACP and PRP-GPS from 1 donor each. MMP-2, -3, and -9 concentrations were assayed in culture media at 24 and 48 h after exposure. | Concentration-(endogenous and total active) and time-dependent release of MMP-2, -3, and -9 from PRP. MMP-2, -3, and -9 expression comparing IL-1β treatment in experimental groups. | • PRP-GPS had higher total MMP-2, -3, and -9 concentrations for up to 144 h of release, while PRP-ACP had higher platelet-normalized MMP-2 and -3 concentrations. PRP-GPS had higher total and endogenous MMP-2, -3, and -9 activity vs PRP-ACP.<br>• ACP and GPS MMP activity was similar. Compared with controls, cells stimulated with IL-1β and treated with ACP showed higher fold changes of MMP-2 and -3 concentration at 24 h than did cells treated with GPS. Total MMP-9 content was higher in the media of GPS-treated IL-1β-stimulated cells vs ACP-treated cells. At 48 h, GPS-treated IL-1β-stimulated cells showed higher fold changes of MMP-2 concentration vs controls, but no difference in MMP-3 concentration was found. At 48 h, there was a higher concentration of MMP-9 in the cell culture media of ACP-treated vs GPS-treated cells.<br>• After exposed to human ligament fibroblasts, both ACP and GPS caused the fibroblasts to release MMPs, highest at 24 h after PRP exposure and dependent on prior IL-1β stimulation.<br>(ACP had lower platelet concentration, lower WBC concentration, and lower hematocrit concentration vs GPS.) |
| Schnabel (2008)[56] | Blood centrifuged at 400*g* for 10 min. | Platelet count: 99,999/μL | Suspensory ligament cultured in plasma, blood, PRP, PPP, and ABM were evaluated for gene expression. DNA analysis and total collagen. | RT-PCR for COL1A1, COL3A1, COMP, MMP-3, MMP-13, and decorin. DNA analysis for viability. Collagen content assessed via Sircol assay. | No significant changes in gene expression, DNA, or collagen observed vs the other conditions tested. |

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Table A2 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Smith (2006)[57] | Blood centrifuged at 150*g* for 15 min at 40°C. PRP prepared by isolating and transferring supernatant. | Platelet count: 522,000/μL | Suspensory ligaments cultured in media with PRP | ELISA for COMP and H-leucine. | Acellular bone marrow treated with PRP had the highest expression of COMP and lowest remaining amount of H-leucine, representative of high protein synthesis. |
| Yoshida (2013)[65] | Porcine whole blood centrifuged at 150*g* for 6 min. First supernatant isolated, centrifuged at 1000*g* for 10 min. Second supernatant isolated were saved (PPP). Pellet resuspended and concentrated with PPP to yield a PRP solution. | • Baseline platelet count: 181 × $10^6$/mL<br>• PRP platelet count: 911 × $10^6$/mL<br>• PMBC cell count: 5 × $10^6$/mL | Porcine ACL fibroblast cultured in PRP ± PBMC | MTT assay, RT-PCR for COL1A1, COL3A1, Sircol collagen assay and ELISA for IL-6. | PRP in conjunction with PBMC led to increased collagen expression, total collagen, IL-6 proinflammatory cytokines, and metabolic activity. |
| Yoshida (2014)[64] | Same as above. Diluted differently to yield 3 different solutions. | • Baseline platelet count: 122 × $10^6$/mL<br>• PRP 1× platelet count: –129 × $10^6$/mL<br>• PRP 3× platelet count: –370 × $10^6$/mL<br>• PRP 5× platelet count: –615 × $10^6$/mL | Porcine ACL explants and exposed to 1×, 3×, and 5× PRP | MTT assay, TUNEL, and RT-PCR for COL1A1 COL3A1, and GAPDH. | PRP 1× yielded better cell viability and collagen production when compared with PRP 3× and PRP 5×. |

[a]See Appendix Table A1 for further abbreviation expansions. ABM, autologous bone marrow; ACL, anterior cruciate ligament; ACP, autologous conditioned plasma; BMA, bone marrow aspirate; COL3A1, Collagen Type III Alpha 1 Chain; COMP, cartilage oligomeric matrix proteins; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GAG, glycosaminoglycan; GPS, GPS III (Biomet); IMHC, immunohistochemistry; MTT - 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide; PBMC, peripheral blood mononuclear cells; PRP, platelet-rich plasma; PRP-LR, platelet-rich plasma that is leukocyte-rich; PRP-PP, platelet-rich plasma with pure platelets; PPP, platelet-poor plasma; RBC, red blood cell; RT-PCR, reverse transcriptase polymerase chain reaction; TEM, transition electron microscopy; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; WBC, white blood cell; WST, Water Soluble Tetrazolium salts.

[b]All cytology values are reported as mean + SD when available.

[c]ADIVA 2120i System, Siemens Healthineers.

TABLE A3
Summary of Included In Vivo Ligament Studies[a]

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Agir (2017)[1] | 8 mL of blood was collected from the marginal ear vein and transferred to Regen THT tube with thixotropic gel to separate RBCs from whole blood. The samples were centrifuged at 3400 rpm for 8 min. PRP was extracted using the syringe. | Not provided | 10 rabbits were placed into 2 groups, 5 rabbits in each group: (1) right extremities of rabbits with tendon-bone integration strengthened via PRP or (2) control group with left extremities of rabbits with tendon-bone integration without PRP (ACL). | On day 56 postop, rabbits were euthanized, and the portion of the distal femur containing the tunnel was amputated. Histological stains were evaluated. | • Histology showed that with PRP, the integration of tendon in the bone was successful without any necrosis formation in most of the tissues.<br>• In the control group without PRP, the integration was distorted in many zones, and some cystic morphologies were present. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Amar (2015)[2] | 2 mL of blood placed in tubes with 3.8% sodium citrate centrifuged at 1500 rpm for 10 min. Supernatant isolated and centrifuged at 3000 rpm for 10 min. Supernatant removed, and pellet resuspended. | Platelet count: 2,750,000/mL | Rat MCL transection model with treatment with PRP and contralateral leg treated as a control. Gross inspection, biomechanical testing, and histology for evaluation at 3 wk. | Biomechanical parameters included load to failure, stiffness, and displacement. Histology evaluated on scale for cellularity, collagen, and vascular. | No difference was found between control and PRP in biomechanical testing and histology. |
| Biercevicz (2013)[3] | <18 g of whole blood placed into tubes and centrifuged at 150*g*. Supernatant collected and then centrifuged at 500*g* to form a platelet pellet that was resuspended in PPP (second supernatant) to concentrate PRP. | PRP platelet count: 1141 ± 527 K/μL | 2 experiments: (1) pigs underwent ACL transection and received either ACL reconstruction, ACL reconstruction with collagen-platelet composite, or no treatment. (2) Pigs underwent ACL transection and received ACL reconstruction, ACL reconstruction with CPC, bioenhanced ACL primary repair with CPC, or no treatment. The surgical legs were harvested after 15 and 52 wk, respectively. | Volume of transected ligaments, max failure load, yield load, linear stiffness, grayscale value from 3D model generation via MRI. | Volume significantly predicted the structural properties (maximum load, yield load, linear stiffness) of the ligaments and grafts. The median grayscale values significantly predicted the structural properties of the ligaments and grafts. |
| Bozynski (2016)[5] | Venous blood obtained via aseptic jugular venipuncture to yield 2 mL of ACP. | Not provided | ACL injury model in dogs (sham, exposed ACL, and partial tear) and treated with NSAIDs, washout, or PRP. | Orthopaedic examination included ROM, pain effusion, lameness, and function. Radiographs obtained. Synovial fluid was analyzed for MCP-1, IL-8, KC, MMP-1, MMP-2, and MMP-3. Gross assessment and scoring system for histology. | On clinical examination, PRP group had better functional assessment. ACL treated was superior in gross appearance. No significance in fluid markers and histology. |
| Cook (2016)[13] | 19-g butterfly catheter with an ACP syringe and without ACDA was used to obtain 15 mL of whole blood from jugular venipuncture. Centrifuged at 1500 rpm for 5 min. | • ACP platelet count in whole blood: 2.5 billion (range, 1.9-2.8 billion)<br>• ACP platelet count in white blood: 296 million (range, 180-400 million) | Canine ACL injury model in partial ACL transection and meniscal release and treated with either PRP injection or saline. | Orthopaedic examination included CROM, pain effusion, lameness, and function. Biomechanical testing for strength and stiffness. Histological evaluation. | Only on orthopaedic examination did PRP yield significant pain reduction and improved ROM. No other significance. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Costa (2017)[14] | 4 mL of blood obtained via central auricular artery punctures and added to 0.3 mL of ACDA-citrate dextrose. Samples homogenized and processed with Cell-DynRuby device for automated platelet quantification. Samples then spun at 800 rpm for 8 min, and supernatant transferred and spun at 3200 rpm for 15 min. Upper two-thirds transferred, and platelets resuspended. | Platelet counts:<br>• Groups 1 and 3: not reported<br>• Group 2: 311.88 ± 81.67 $mm^3$ baseline; PRP: 3.32 ± 0.89 times baseline<br>• Group 4: 323.87 ± 66.83 $mm^3$ baseline; PRP: 3.33 ± 0.79 times baseline | 30 New Zealand White rabbits subjected to MCL tears and assigned to 1 of 4 groups: (1) saline treatment at 3 wk, (2) PRP treatment at 3 wk, (3) saline treatment at 6 wk, (4) PRP treatment at 6 wk. | Biomechanical testing of ligament strength between PRP and control group. | PRP significantly increased ligament tensile strength at 3 and 6 wk vs control. |
| Fleming (2009)[23] | Blood centrifuged at 150*g*. Supernatant isolated and centrifuged at 500*g*. Second supernatant isolated as PPP. Pellet resuspended PPP to create PRP. | Platelet count: 1141 ± 527 × 1000/μL | Porcine ACL model treated with PRP-collagen graft compared with untreated graft. Evaluated at 15 wk. | Orthopaedic examination (maximum flexion and extension) via gross inspection. Biomechanical testing of AP laxity at 30°, 60°, and 90°; tensile load displacement; tangent modulus; linear stiffness; and yield stress. | No significant difference visually or via orthopaedic examination. Biomechanical properties were significant for yield and max failure loads. Central necrosis observed in the non-PRP group on histology. |
| Fleming (2015)[22] | Blood centrifuged at 150*g*. Supernatant isolated and centrifuged at 500*g*. Second supernatant isolated as PPP. Pellet resuspended PPP to create PRP solution (1×, 3×, 5×). | • PRP 1x platelet count: 466/μL (387.3/mL-544.7/μL)<br>• PRP 3x platelet count: 1386/μL(1153/μL-1618/μL)<br>• PRP 5x platelet count: 2473/μL (2011/μL-2937/μL) | Porcine ACL transection in model and subsequently repaired with ECM scaffold platelet concentrate at 1×, 3×, and 5× PRP. Evaluated at 15 wk. | Biomechanical testing; AP knee laxity measured at 30°, 60°, and 90° of flexion and structural properties. Histology assessed. | Linear stiffness was significant in the 1× preparation. No other results significant. |
| Harris (2012)[26] | 50 mL of phlebotomized blood combined with citrate dextrose solution and loaded into a Platelet Concentrate Collection System tube (Implant Innovations). Centrifuged at 3000 rpm for 3 min 45 s. Plasma layer isolated and transferred, centrifuged at 3000 rpm for 3 min 45 s. Plasma layer | Platelet count: 1,348,667 ± 427,278/μL | PRP was injected into the soft tissues of a rabbit in the MCL to assess PRP in healthy tissue. | Histological assessment of the various rabbit tissues exposed to PRP. | On histology, the ligament showed inflammation that continued to be evident at 12 wk. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| | isolated and transferred, centrifuged at 3000 rpm for 13 min. PPP removed. Pellet resuspended in PPP to 2.5 mL and activated via 0.5-mL mixture of topical bovine thrombin (5000 U) and $CaCl_2$ (30 mL) to yield PRP gel (3 mL). | | | | |
| Haus (2012)[27] | 60 mL of whole blood was drawn and centrifuged at 150*g* then centrifuged again at 500*g* to form a platelet pellet. Pellet was resuspended with PPP to make 5× whole-blood platelets concentration. | 1st study platelet counts:<br>• Immature group: PRP = 1914 ± 140 × $10^3$/mL<br>• Adolescent group: PRP = 1779 ± 140 × $10^3$/mL<br>• Adult group: PRP = 1443 ± 177 × $10^3$/mL<br>2nd study platelet counts:<br>• Immature group: Systemic = 412 ± 127 × $10^3$/μL; PRP = 736 ± 236 × $10^3$/μL<br>• Adolescent group: Systemic = 318 ± 132 × $10^3$/μL; PRP = 529 ± 210 × $10^3$/μL<br>• Adult group: Systemic = 304 ± 102 × $10^3$/μL; PRP = 615 ± 147 × $10^3$/μL | 3 groups for ACL transection: skeletally immature (juvenile), adolescent, and adult. In the 1st experiment, 4 pigs from each group underwent bilateral transection. One knee was treated with PRP, and 1 was not (control, knee was simply opened and then closed). In 2nd experiment, both sides in each group were treated with bioenhanced suture repair. Insertion sites in all were assessed. | Histological response of the insertion site at 1, 2, 4, and 15 wk; biomechanical healing (strength, max load); collagen organization; cellularity; qualitative morphology; fibroblast density; osteoclast density; and blood vessel density. | • In young and adolescent animals treated with bioenhanced suture repair with CPC, changes in the insertion site included (1) fibroblastic proliferation with loss and return of collagen alignment in the fibrous zone, (2) osteoclastic resorption within fibrocartilage zones at 2-4 wk, and (3) partial reappearance of fibrocartilage zones at 15 wk.<br>• In adult animals, degenerative changes were noted by wk 15: (1) loss of parallel arrangement of collagen fibers in the fibrous zone and (2) increasing disorganization and loss of columniation of chondrocytes in the fibrocartilage zone. |
| Joshi (2009)[29] | CPC: blood was drawn from the pig before surgery and centrifuged for PRP. Neutralized collagen and PRP were mixed 1:1 to form CPC. | PRP platelet concentration: 1,279,000 ± 775,000 platelets/$mm^3$ | 27 knees in immature pigs underwent ACL transection and suture repair. CPC was used to supplement the repair in 14 knees. | Biomechanical testing (yield load, stiffness, yield displacement, cellularity, cell shape, cell orientation, vascularity, collagen density), gross observations (shape, scar mass, noted repair tissue), scar mass size on | CPC group had more scar mass at 3 mo. No difference in hypertrophic repair tissue, max cross-sectional area, cellularity, or vascularity. CPC group had significant improvements in yield load and linear |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| | | | | MRI scan histomorphometry (average cellularity, alignment, vascularity, ligament characteristics, cell density, shape) | stiffness of the repair tissue at 3 mo, as well as a significant increase in cell density. Both groups had a reduction in yield load and stiffness at 6 wk, noted with high vascularity. |
| LaPrade (2018)[31] | Blood from each animal spun at 400*g* for 5 min, and plasma transferred. For PPP and PRP, sample spun at 1000*g* for 5 min. For PRP samples, PPP supernatant removed, and 1 mL of PPP used to suspend pellet. | PPP platelet count:<br>• PRP 2×: 0.6 million/μL.<br>• PRP 4×: 1.2 million/μL.<br>• Mean WBC count in PRP samples was $0.3 \times 10^3/\mu L \pm 0.3 \times 10^3/\mu L$ | New Zealand White rabbits with grade 3 MCL tears were administered PPP, PRP 2×, and PRP 4× and compared with control saline injection and with sham surgery groups. | Collagen subscore, vascularity subscore, ligament tissue maturity index score, maximum load, knee stiffness. Gross and histological assessments. | PPP and PRP 4× groups had significantly lower collagen subscores than control had; PRP 4× treatment resulted in significantly less ligament strength than did control. All treatment groups were significantly inferior to the sham surgery group in maximum load, displacement at maximum load, and work to maximum load and had less knee stiffness. All treatment groups had lower vascularity subscores and ligament tissue maturity index score than did sham surgery group. No macroscopic inflammation in any knees. MCL width was not statistically significant among groups. |
| Lee (2012)[32] | Blood centrifuged at 1210*g* for 10 min. Plasma isolated and centrifuged at 2422*g* for 20 min at 4°C. Supernatant isolated and discarded to yield PRP (1 mL). 60 μL of 2500 IU/mL solution (1 mL of calcium gluconate mixed with 5000 IU/mL of bovine thrombin) mixed with remaining PRP (540 μL) via dual-syringe system to yield 10:1 ratio. | Not provided | ACL rabbit model treated with canine small intestinal submucosal grafts with and without treatment of PRP. Evaluated for gross, histological, and biomechanical repair. | ROM, gross, and histological analysis. Biomechanical testing for maximal stress, strain, and ultimate load. | Over 7 days, TGF-β release decreased as exogenous source depleted. PRP group had superior ROM at 2, 4, and 6 wk. The control group had significantly improved biomechanical results compared with PRP. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Mastrangelo (2011)[39] | PRP made with 5× and 3×platelet count; the baseline of platelets was not specified. | • Systemic platelet count: $391 \pm 48 \times 10^9$/L<br>• PRP 5× platelet count: $1951 \pm 304 \times 10^9$/L<br>• PRP 3× platelet count: $1161 \pm 179 \times 10^9$/L | 2 randomized groups of 8 animals (for bilateral ACL transection and suture repair) with 1 knee receiving 5× baseline systemic platelet count and 1 knee receiving 3×. | Systemic platelet count, concentrated platelet counts, anteroposterior laxity, tensile testing, cellular density within the ACL, cell orientation and shape, collagen formation, and maturity index of wound area. | The decrease in platelet concentration from 5× to 3× to enhance suture repair of the ACL did not significantly harm the mechanical outcomes in this animal model. Femoral insertion site and central wound site had significant decreases in cellular density at 13 wk postop. 3× group showed more areas of disorganization and defects in terms of cell shape and collagen formation. 5× group had higher cellularity and vascularity subscores in terms of maturity index of wound area. |
| Matsunaga (2013)[40] | Blood centrifuged 3000 rpm for 15 min at 4°C. Supernatant centrifuged at 3000 rpm for 15 min at 4°C. Platelet-poor layer and platelet/leukocyte layer frozen at –80°C. Platelet-poor layer defrosted and ultrafiltrated twice at 4°C (Vivaflow50 MW10000; Vivascience). Enriched layer and defrosted platelet/leukocyte layer mixed with calcium gluconate to 23 milliMolar. Mixture incubated at 37°C for 3 h to produce PRP gel. | Not provided | Rabbit MCL model treated with PRP turned into compact platelet-rich fibrin scaffold and evaluated at 4, 8, and 12 wk for histology and gross appearance and 20 wk for biomechanics. | Assessed gross appearance, histology, and mechanical properties of ultimate load and stiffness. | Macroscopically and microscopically treated groups were superior. Biomechanical properties were unable to be collected for control provided the tissue. PRP-treated yield results slightly below those of healthy control. |
| Murray (2006)[49] | Blood centrifuged at 100*g* for 20 min. 300 μL of isolated PRP added to acid-soluble type I collagen (700 mL). | Not provided | Canine ACL model treated with PRP. Evaluated via histology and biomechanical properties at 3 and 6 wk. | IMHC for fibrinogen, fibrin, and fragments. Mechanical testing for ultimate load, work to failure, and stiffness. | Significant ACL filling in PRP group. Significant increase in ultimate load and work to failure at 6 wk vs control. No difference in stiffness. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| Murray (2007)[48] | Blood centrifuged at 100*g* for 20 min. Supernatant transferred, leaving PRP. 300 μL of isolated PRP added to acid-soluble type I collagen (700 mL). | Not provided | Canine ACL model assessing intra-articular ACL treated with PRP vs extra-articular MCL and patellar ligament and evaluated via histology at multiple time points. | IMHC for FGF-2, TGF-β1, PDGF-A, von Willebrand factor, procollagen, fibrinogen, and fibronectin. Histological scoring for cellularity, collagen, and vascularity. | Wound healing differed between extra-articular MCL/patellar ligament and intra-articular ACL. When PRP was applied, ACL healing statistically improved but still differed from MCL/patellar ligament healing. PRP increased levels of fibrinogen, fibronectin, PDGF-A, TGF-β1, FGF-2, and von Willebrand factor. |
| Murray (2007)[47] | 9 mL of blood was obtained from phlebotomy and centrifuged at 100*g* for 14 min, and PRP isolated. | Platelet count range: 780,000-2,300,000/mm$^3$ | Porcine ACL model treated with PRP collagen and compared with suture alone. Evaluated at 4 wk for biomechanical properties and histology. | Biomechanical properties including load at yield, maximum load, displacement at yield, displacement at failure, tangent modulus, and energy at failure. MRI and histology comparison. | PRP led to significant improvement and difference in load at yield, maximum load, tangent modulus, and energy to failure compared with suture alone. |
| Murray (2009)[46] | 60 mL of whole blood mixed with 6 mL of acid-citrate dextrose in tubes. Centrifuged at 1200*g* for 4 min. 7 mL of top layer removed, and 2 mL of acid-citrate dextrose added. Centrifuged at 1000*g* for 9 min. Supernatant removed, and pellet resuspended in 10 mL of supernatant. Mixture centrifuged at 1200*g* for 2 min. 7 mL of PRP isolated. | Not provided | Porcine ACL tear model treated with PRP and suture vs suture alone. Evaluated at 14 wk for biomechanical properties. | Gross inspection of ACL. AP laxity at 30° and 60°. Mechanical testing of linear stiffness, maximum tensile load, displacement at failure, and energy at failure. | No significant difference |
| Murray (2013)[45] | Not provided | Not provided | 64 pigs underwent ACL transection and were randomly placed into 4 experimental groups: (1) no treatment, (2) conventional ACL reconstruction with BPTB allograft, (3) bioenhanced ACL reconstruction with BPTB allograft and a | Linear stiffness, yield load, max load, cross-sectional area, AP laxity, histology, total lesion area of cartilage | Linear stiffness, yield load, and max load after bioenhanced ACL repair were not significantly different from those after bioenhanced or conventional ACL reconstruction but were significantly greater than those after untreated ACL transection after 12 mo of healing. Only mean AP |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| | | | bioactive scaffold, and (4) bioenhanced ACL repair using a bioactive scaffold | | laxity value for ACL reconstruction group was significantly lower than that for ACL-transected group at 6 mo. At 12 mo, all mean AP laxity values at 30° of flexion for surgical treatment were significantly lower than those for nontreated group. Macroscopic cartilage damage after bioenhanced ACL repair was significantly less than that after untreated ACL transection and bioenhanced ACL reconstruction, with a strong trend that it was less than that after conventional ACL reconstruction in the porcine model at 12 mo. |
| Nin (2009)[50] | 40 mL of blood drawn from patient 1 h preop and centrifuged for 8 min at 3000 rpm. The blood cell component of the fraction was separated and centrifuged for 6 min at 1000 rpm at room temperature. Filtrate obtained to yield PRP. 0.05 mL of $CaCl_2$ added intraop to activate platelets. | Platelet count: 837 × $10^3$/$mm^3$ | 2 randomized groups of 50 patients undergoing ACL reconstruction: patellar tendon allograft reconstruction with platelet-enriched gel and without the gel. | VAS pain, anterior laxity, and IKDC score at 1 d postop. C-reactive protein at 1 and 10 d postop. difference in perimeter in the middle and 5 cm above top edge of the kneecap preop and 1 d postop. Anterior tibial displacement, tunnel placement, and graft position in the tibia and femur. Intensity, uniformity, and thickness at center of graft and PCL angle. | No differences in the number of associated injuries. No significant differences between the groups for inflammatory parameters (perimeters of the knee and C-reactive protein level), MRI appearance of the graft, and clinical evaluation scores (VAS, IKDC, and KT-1000 arthrometer). |
| Prządka (2017)[52] | Tray Life Set (Croma-Pharm) for PRP preparation. 4.5 mL of venous blood extracted from marginal ear vein rabbits and transferred to tubes with sodium citrate. Centrifuged at 100*g* for 10 min. Serum extracted and centrifuged at 340*g* for 10 min. Top layer removed to yield 1 mL PRP. | Platelet count: 536-1909 g/L | Polyester implants placed in 32 New Zealand rabbits for repair of the cranial cruciate ligament were saturated with PRP, antlerogenic stem cells MIC-1, and their homogenate prior to surgery. | Morphological and biochemical blood tests, total protein and proteinogram, concentrations of acute phase proteins (fibrinogen and haptoglobin), gross assessment of surgical region, and histological assessment of surgical region. | No statistical differences in morphological parameters. All groups had lower mean concentrations of protein PRP and increased fibrinogen content. PRP group had lower mean haptoglobin concentration than did antlerogenic stem cells MIC-1 group (group 2). |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
|---|---|---|---|---|---|
| | | | | | Histological examination showed no inflammatory reaction, and PRP increased connective tissue that wrapped around/through polyester cranial cruciate ligament implant. |
| Sample (2018)[54] | 2 mL of blood drawn for platelet count, and 32 mL of blood drawn for PRP and centrifuged at 100 *g* for 14 min. PRP was then extracted. | Mean platelet count: 3.6 ± 0.23 mL (range, 3.1-4.2 mL) | 29 dogs with unilateral complete CR and contralateral partial CR divided into 2 groups: (1) Complete CR dogs were treated with tibial plateau - leveling osteotomy. (2) Contralateral partial CR dogs were treated with PRP-collagen scaffolds. Dogs were evaluated at 10 wk and 12 mo after treatment. | Correlation between both development of complete CR and time to complete CR with diagnostic tests including bilateral stifle radiographs, MRI, and bilateral stifle arthroscopy. Histological evaluation of synovial biopsies, C-reactive protein concentrations in serum and synovial fluid, and synovial total nucleated cell count. | Single application of PRP collagen in partial CR stifles of dogs was not an effective disease-modifying therapy for the prevention of progression to complete CR. Radiographic effusion, arthroscopic evaluation of cranial cruciate ligament damage, and MRI assessment of ligament fiber tearing in partial CR stifles correlated with progression to complete CR over the 12-mo follow-up period. Noted that the best predictive model for development of complete CR in PRP-collagen-treated partial CR stifles included variables from multiple diagnostic modalities. |
| Sánchez (2010)[55] | PRGF: 65 mL of peripheral venous blood was drawn into 9-mL tubes. Tubes were centrifuged at 580 *g* for 8 min at room temperature. (Fibrin was harvested from the plasma.) 2-mL plasma fraction was collected and had moderate platelets. | Cytology not provided, but authors mentioned that platelet count was 2- to 3-fold of peripheral blood. | 2 groups for ACL repair: control group (conventional reconstruction) and PRGF-assisted ACL reconstruction with autogenous hamstring; required 2nd-look arthroscopy to remove hardware or loose bodies, treat meniscal tears or plica syndrome, or resect cyclops lesions at 6 to 24 mo postop. | Arthroscopic score (0-4 points) evaluating graft thickness/ apparent tension (0-2 points) plus synovial coverage (0-2 points). Histological transformation of the tendon graft to ACL-like tissue was evaluated by use of the Ligament Tissue Maturity Index, and a score to assess the progression of new connective tissue enveloping the graft | Arthroscopic evaluations were not statistically different between PRGF and control groups. PRGF treatment influenced the histological characteristics of the tendon graft, resulting in tissue that was more mature than that in controls. Histologically evident, newly formed connective tissue enveloping |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
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| | | | | was created by use of 3 criteria previously used to characterize changes during ligament healing: cellularity, vascularity, and collagen properties. | the graft was present in 77.3% of PRGF grafts and 40% of controls. The appearance of the connective tissue envelope changed with increasing time from surgery. The remodeling of PRGF grafts involved the formation of synovial-like tissue enveloping the graft, which integrated in the remodeled tendon graft to look similar to a normal ACL. |
| Smith (2020)[58] | Not provided | Not provided | • 2 groups of dogs underwent arthroscopic complete transection of the ACL, which was repaired via either arthroscopic-assisted all-inside ACL reconstruction using the QTIB allograft or a BPTB autograft. Contralateral knees were used as nonoperated controls.<br>• QTIB ACL grafts were soaked in autogenous PRP. | • Radiographic assessments were performed at 2 mo.<br>• Arthroscopic assessments were performed at 6 mo.<br>• Anterior drawer, internal rotation, lameness, kinetics, pain, effusion, and comfortable knee ROM were measured at 2, 3, and 6 mo.<br>• Biomechanical and histological assessments were performed at 6 mo. | At 6 months, QTIB-reconstructed knees had significantly less lameness, lower pain, less effusion, and increased ROM vs BPTB knees. BPTB knees had significantly higher radiographic OA scores than did QTIB knees at 6 mo. PRP-QTIB showed overall better outcomes. |
| Teng (2016)[59] | 10 mL of whole blood was drawn from the marginal auricular vein. It was centrifuged at 1200*g* for 10 min and centrifuged again at 2500*g* for 20 min at 4°C. 1 mL of PRP was collected. The precipitated platelets (1 mL) were collected. | Not provided | 3 groups for ACL reconstruction using hamstring tendon: control group, PRP group, and BMSC+PRP group | Expression of CD44, CD45, and CD90. Transcription of collagen I, osteocalcin, and osteopontin (oncogenes). Histological observations and micro-CT scan conducted. Biomechanical testing (failure load, stiffness). | Collagen I, osteocalcin, and osteopontin expression was higher in BMSCs cocultured with PRP for 14 d. More mature tendon–bone interface using light microscopy, more newly formed bone at the bone tunnel walls detected via micro-CT, and a significantly higher failure load as assessed via biomechanical testing in the BMSC+PRP group than in the control and PRP groups. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
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| Valenti Azcarate (2014)[60] | • Method 1: 40-mL sample of citrated blood per patient centrifuged at 3000*g* for 8 min. Between the serum and blood-cell phases, pipetted out and centrifuged at 380*g* for 6 min. Filtrate obtained to yield PRP. 0.05 mL of $CaCl_2$ added intraop to activate platelets.<br>• Method 2: centrifuged at 580*g* for 8 min at 1800 rpm. Middle section collected, and 0.05 mL of 10% $CaCl_2$ added. | • Baseline platelet count: 201 × $10^6$/mL<br>• PRP double-spin: platelet count = 837 × $10^6$/mL<br>• Mean leukocyte concentration = 8100/μL<br>• PRGF: platelet count = 242 × $10^6$/mL<br>• Plasma without leukocytes: platelet count = 504 × $10^6$/mL | 3 groups: (1) control (non-gel), (2) PRP with leukocytes via double-spinning procedure, and (3) PRP via single spinning procedure without leukocytes. | C-reactive protein, knee perimeters (difference in perimeter in the middle and 5 cm above top edge of the kneecap preop and 1 d postop). VAS score 1 d postop. IKDC at 3, 6, and 12 mo. Radiology results included thickness, intensity, uniformity, tunnel direction, tibial anterior translation, and position of PCL. | The PRGF group showed significant improvement in swelling and inflammatory parameters vs the other 2 groups at 24 h postop. The results did not show any significant differences between groups for MRI and clinical scores. |
| Xie (2013)[62] | 2 tubes centrifuged at 200*g* for 10 min (room temperature). PPP layer and PRP layer isolated and transferred to 2 new tubes. Tubes centrifuged at 200*g* for 10 min. PPP supernatant removed to yield 1 mL of PRP. | Platelet count: 669 ± 51 × $10^9$/L | Canine (beagle) model of ACL reconstruction surgery with treatment of PRP. Evaluated gene expression. | RT-PCR for COL1A1, COL3A1, TGF-β1, decorin, biglycan, MMP-1, MMP-13, TIMP-1, and GAPDH assessed at 2, 6, and 12 wk. | For PRP groups COL1A1 peaked at 6 wk and COL3A1 peaked at 2 and 6 wk. Time-dependent changes in expressed genes. |
| Xie (2013)[63] | 2 tubes centrifuged at 200*g* for 10 min (room temperature). PPP layer and PRP layer isolated and transferred to 2 new tubes. Tubes centrifuged at 200*g* for 10 min. PPP supernatant removed to yield 1 mL of PRP. | Platelet count: 669 ± 51 × $10^9$/L | Canine (beagle) model of ACL reconstruction surgery with treatment of PRP. Evaluated gene expression. | RT-PCR for CD31, VEGF, thrombospondin 1, neutrophil 3, growth-association protein 43, and nerve growth factor assessed at 2, 6, and 12 wk. | Time-dependent changes in expressed genes. |
| Yoshioka (2013)[66] | Four 5-mL sterile tubes with 3% trisodium citrate centrifuged at 460*g* for 8 min. PPP, PRP layer, and last 500 μL above erythrocyte layer isolated. 500-μL layer activated using 10% $CaCl_2$. | Platelet count: 39.5 ± 2.4 × $10^4$/μL | Rabbit MCL tear model, treated with PRP-Endoret and evaluated at 3 and 6 wk via gross inspection, histology, and mechanical strength. | Gross inspection. Histological assessment for cellularity, blood vessel density, and collagen. Biomechanical analysis for max load and stiffness. | Histologically. PRP resulted in increased cellularity at 3 wk, and alignment of collagen fibers was noted. PRP also had statistically better performance on load and stiffness. |

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Table A3 (continued)

| Lead Author (Year) | PRP Preparation | Cytology Findings[b] | Study Design | Outcomes Measured | Results |
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| Zhang (2019)[67] | PRP: 10 mL of peripheral blood collected from the marginal auricular vein before the surgery. Blood centrifuged at 300*g* for 10 min. Plasma collected into another tube and centrifuged at 400*g* for 20 min at 4°C. 1 mL of PRP collected. | Platelet count: 151.0 ± 38.3 × $10^6$/mL | 3 groups of rabbits underwent semitendinosus autograft ACL reconstruction: without PRP, with PRP, and with PRP-GS. | Scaffold characterization (pore sizes, weight), rate of TGF-β1 release, optical density, expression of tendon-to-bone healing-related genes, and MRI signal. Biomechanical tests including failure load, stiffness. Amounts of inflammatory synovium. Histologically relative observations and scoring. | Levels of related genes were upregulated in PRP-GS group compared with PRP group. Lower signal on MRI scan indicated fibrocartilage formation in the 2 groups with PRP. Histological staining showed that the tendon-bone connection had a greater fibrocartilaginous transition region in the PRP-GS group, and the histological scores were higher compared with the PRP group. Maximum failure load and stiffness were higher in the PRP-GS group than in the other 2 groups. |

[a]See Appendix Table A1 for further abbreviation expansions. 3D, 3-dimensional; AP, anteroposterior; ACDA, anticoagulant citrate-dextrose solution A; ACL, anterior cruciate ligament; ACP, autologous conditioned plasma; BMSC, bone marrow mesenchymal stem cells; BPTB, bone–patellar tendon–bone; CPC, collagen-platelet composite; CR, cruciate ligament rupture; CT, computed tomography; ECM, extracellular matrix; FD, freeze-dried; FGF, fibroblast growth factor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GS, gelatin sponge; ICA, icariin; IKDC, International Knee Documentation Committee score; IMHC, immunohistochemistry; intraop, intraoperatively; KC, keratinocyte-derived chemoattractant; MCL, medial collateral ligament; MIC, macrophage inhibitory cytokine; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OA, osteoarthritis; PCL, posterior cruciate ligament; PDGF, platelet derived growth factor; postop, postoperative; PPP, platelet-poor plasma; preop, preoperative; PRGF, plasma rich in growth factor; PRP, platelet-rich plasma; QTIB, quadriceps tendon allograft with internal brace; RBC, red blood cell; ROM, range of motion; RT-PCR, reverse transcriptase polymerase chain reaction; TGF, tumor growth factor; THT, Regen THT system to prepare autologous platelet rich plasma; VAS, visual analog scale; VEGF, vascular endothelial growth factor; WBC, white blood cell.

[b]All cytology values are reported as mean + SD when available.