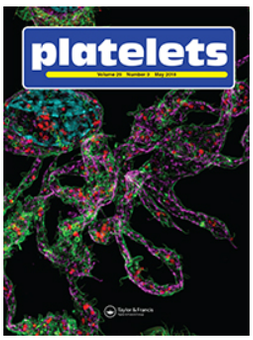

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REVIEW ARTICLE

The use of platelet-rich plasma to treat chronic tendinopathies: A technical analysis

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Abstract

Platelet-rich plasma (PRP) is blood plasma with a high concentration of autologous platelets which constitute an immense reservoir of growth factors. The clinical use of PRP is widespread in various medical applications.

Although highly popular with athletes, the use of PRP for the treatment of tendinopathies remains scientifically controversial, particularly due to the diversity of products that go by the name of "PRP." To optimize its use, it is important to look at the various stages of obtaining PRP.

In this literature review, we take a closer look at eight parameters which may influence the quality of PRP: 1) anticoagulants used to preserve the best platelet function, 2) the speed of centrifugation used to extract the platelets, 3) the platelet concentrations obtained, 4) the impact of the concentration of red and while blood cells on PRP actions, 5) platelet activators encouraging platelet degranulation and, hence, the release of growth factors, and 6) the use or nonuse of local anesthetics when carrying out infiltration. In addition to these parameters, it may be interesting to analyze other variables such as 7) the use of ultrasound guidance during the injection with a view to determining the influence they have on potential recovery.

Keywords

Collection, optimization, platelet-rich plasma, PRP, standardization, tendinopathies

History

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Introduction

Platelet-rich plasma (PRP) consists of blood plasma with a high concentration of autologous platelets which constitute an immense reservoir for growth factors [1,2], as PDGF (Platelet-Derived Growth Factor), IGF-1 (Insuline-Like Growth Factor), TGF-β (Transforming Growth Factor), EGF (Epidermal Growth Factor), VEGF (Vascular Endothelial Growth Factor), etc. These are essential for the initiation and stimulation of the healing mechanism and the synthesis of collagen among other actions [3,4]. The clinical use of PRP is widespread in various areas such as maxillo-facial surgery, sports traumatology, orthopedic surgery, and dermatology [5–7].

The conservative treatment of tendinopathies is difficult, and pain remains often rebel to classic treatments [8,9]. This is why new treatments, including PRP, are currently being assessed. Although highly popular with athletes, however, its use remains scientifically controversial [10–12], particularly of products and kits available in the market as PRP and also due to different methodologies and lack of standardization to obtain PRP [13–15]. To optimize its use, it is important to take a look at the various stages of obtaining PRP [16].

We decided to carry out a literature review and, more specifically, to analyze eight parameters which can influence the quality of PRP: 1) anticoagulants used to preserve the best platelet function, 2) the speed of centrifugation used to extract the platelets, 3) the platelet concentrations obtained, 4) the presence of leukocytes and erythrocytes in the PRP, 5) platelet activators encouraging platelet degranulation and, hence, the release of growth factors, and 6) the use or nonuse of local anesthetics when carrying out infiltration. In addition to these parameters, it may be interesting to analyze other variables such as 7) the use of ultrasound guidance during the injection with a view to determining the influence they have on potential recovery.

Material and methods

A search for articles was conducted in the Pubmed and Pedro databases. The results were obtained by using the following key words and combinations of these key words: PRP, platelet-rich plasma, injection, treatment, tennis elbow, greater trochanteric pain syndrome, jumper's knee, Achilles tendinopathy, plantar fasciitis, anticoagulant, sodium chloride, local anesthetic, and centrifugation speed.

Results

The studies are given in chronological order in the form of summary tables according to the type of tendinopathy treated (Tables I–IV) [17–69].

Anticoagulant

The choice of anticoagulant capable of preserving the greatest platelet integrity and functionality is an important factor. In

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Table I. Various clinical studies using platelet rich plasma to treat epicondylitis.

Study	Anticoagulant used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	Injected volume of PRP	Results
Peerbooms et al. [17] Am J Sports Med 2010	ACD-A (3 ml)	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM–15 min	8.4% sodium bicarbonate	No	1 ml	Reduction in VAS for 49% of corticosteroids and 73% of PRP Reduction in pain and improved function by PRP
Galanis et al. [18] J Bone Joint Surg 2011	ACD-A	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM - 15min	8.4% sodium bicarbonate	No	Not mentioned	Improved VAS (84 to 7) and Mayo score (51 to 92). Reduction in pain and improved function by PRP
Hechtman et al. [19] Orthopedics 2011	Trisodium citrate (1 ml)	Not mentioned	Not mentioned	Calcium chloride (0.1 ml)	1% xylocaine without epinephrine	1100 g–6 min	No	No	3 ml	Improvement in 90% of cases (>25% reduction)
Creaney et al. [20] Br J Sports Med 2011	ACD-A	3×	Not mentioned	No	2 ml bupivacaine	2000 g–15 min	No	Yes	1.5 ml	At six months, satisfaction rate of 66% for the PRP group compared to 72% for the autologous blood injection.
Thanasas et al. [21] Am J Sports Med 2011	ACD-A (3–5 ml)	1 295 500/ml (5,5×)	White blood cells: ratio of 111/1 platelets/ leukocytes)	No	bupivacaine	3200 RPM–15 min	No	Yes	3 ml	Improved VAS score higher for PRP at six weeks only PRP appears to be more effective than autologous blood in the short term
Gosens et al. [22] Am J Sports Med 2011	ACD-A	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM – 15 min	8.4% sodium bicarbonate	No	1 ml	Improved DASH score for 72% (>25% reduction). Improved pain and function
Omar et al. [23] Egypt Rheumatol 2012	Citrate Phosphate dextrose (CPD)	2×	Not mentioned	Not mentioned	Not mentioned	320 g–15 min/2000 g–15 min	Not mentioned	No	Not mentioned	Improved VAS (PRP from 8 to 3.8 and control from 8.6 to 4.3) and DASH (PRP from 58.9 to 19.9 and control from 57.3 to 20.2).
Krogh et al. [24] Am J Sports Med 2013	Sodium Citrate (3 ml)	8×	Not mentioned	No	10–15 ml of Lidocaine 10 mg/mL	3200 g–15 min	8.4% sodium bicarbonate	Yes	3–3.5 ml	Improved PRTEE: PRP (27.5 to 6), saline solution (25 to 3) glucocorticoid (28 to 7.1) Pain reduction PRP > glucocorticoid No difference between groups
Martin et al. [25] Trials 2013	9 ml of 3,8% sodium citrate	Not mentioned	Not mentioned	10% calcium chloride (50 µL/ ml of PRP)	2 ml of 1% lidocaine	1200 RPM – 6 min	No	Yes	3–5 ml	Forthcoming

(*Continued*)

Table I. (Continued).

Study	Anticoagulant used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	Injected volume of PRP	Results
Mishra et al. [26] Am J Sports Med 2013	ACD-A	5×	Not mentioned	No	0.5% bupivacaine	3200 RPM – 15 min	8,4% soidum bicarbonate	No	2-3 ml	82.1% of subjects had more than 50% reduction in pain
Raeissadat et al. [27] BMC Sports Sci Med and Rehab 2014	ACD-A (2 ml)	4.8×	Not mentioned	No	Lidocaine	1600R PM–15 min /2800 RPM - 7min	No	No	2 ml	After 12 months, 75% of subjects had a reduction in pain by >25%
Tonk et al. [28] Indian J of Ortho 2014	ACD-A (3 mg)	5×	Not mentioned	No	1ml of 2% xylocaine	700 RPM – 20 min /1750 RPM - 15 min	No	No	3 ml	Reduction in Nirschl score for both groups Better results in the short term for laser and long term for PRP
Chiavaras et al. [29] Acad Radiol 2014	No	Not mentioned	Not mentioned	No	1% lidocaine	1500 RPM – 5 min	No	Yes	3 ml	Forthcoming
Ford et al. [30] American Association for Hand Surgery 2014	ACD-A (1 ml)	Not mentioned	Not mentioned	Not mentioned	5 ml of 1% lidocaine	1500 RPM – 5 min	Not mentioned	No	3-4 ml	Reduction in pain for 89.3%

Table II. Various clinical studies using platelet rich plasma to treat patellar tendinopathies.

Study	Anticoagulant used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	Injected volume of PRP	Results
Peerbooms et al. [17] Am J Sports Med 2010	ACD-A (3 ml)	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM–15 min	8.4% sodium bicarbonate	No	1 ml	Reduction in VAS for 49% of corticosteroids and 73% of PRP Reduction in pain and improved function by PRP
Galanis et al. [18] J Bone Joint Surg 2011	ACD-A	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM–15 min	8.4% sodium bicarbonate	No	Not mentioned	Improved VAS (84 to 7) and Mayo score (51 to 92). Reduction in pain and improved function by PRP
Hechtman et al. [19] Orthopedics 2011	Trisodium citrate (1 ml)	Not mentioned	Not mentioned	Calcium chloride (0.1 ml)	1% xylocaine without epinephrine	1100 g–6 min	No	No	3 ml	Improvement in 90% of cases (>25% reduction)
Creaney et al. [20] Br J Sports Med 2011	ACD-A	3×	Not mentioned	No	2 ml bupivacaine	2000 g–15 min	No	Yes	1.5 ml	At six months, satisfaction rate of 66% for the PRP group compared to 72% for the autologous blood injection.
Thanasas et al. [21] Am J Sports Med 2011	ACD-A (3–5 ml)	1 295 500/ml (5,5×)	White blood cells: ratio of 111/1 platelets/ leukocytes)	No	bupivacaine	3200 RPM–15 min	No	Yes	3 ml	Improved VAS score higher for PRP at six weeks only PRP appears to be more effective than autologous blood in the short term
Gosens et al. [22] Am J Sports Med 2011	ACD-A	Not mentioned	Not mentioned	No	0.5% of bupivacaIne with epinephrine	3200 RPM–15 min	8.4% sodium bicarbonate	No	1 ml	Improved DASH score for 72% (>25% reduction). Improved pain and function
Omar et al. [23] Egypt Rheumatol 2012	Citrate Phosphate dextrose (CPD)	2×	Not mentioned	Not mentioned	Not mentioned	320 g–15 min /2000 g–15 min	Not mentioned	No	Not mentioned	Improved VAS (PRP from 8 to 3.8 and control from 8.6 to 4.3) and DASH (PRP from 58.9 to 19.9 and control from 57.3 to 20.2).
Krogh et al. [24] Am J Sports Med 2013	Sodium Citrate (3 ml)	8×	Not mentioned	No	10–15 ml of Lidocaine 10 mg/mL	3200 g–15 min	8.4% sodium bicarbonate	Yes	3–3.5 ml	Improved PRTEE: PRP (27.5 to 6), saline solution (25 to 3) glucocorticoid (28 to 7.1) Pain reduction PRP > glucocorticoid No difference between groups
Martin et al. [25] Trials 2013	9 ml of 3,8% sodium citrate	Not mentioned	Not mentioned	10% calcium chloride (50 µL/ ml of PRP)	2 ml of 1% lidocaine	1200 RPM–6 min	No	Yes	3–5 ml	Forthcoming

(*Continued*)

Table II. (Continued).

Study	Anticoagulant used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	Injected volume of PRP	Results
Mishra et al. [26] Am J Sports Med 2013	ACD-A	5×	Not mentioned	No	0.5% bupivacaine	3200 RPM–15 min	8,4% soidum bicarbonate	No	2-3 ml	82.1% of subjects had more than 50% reduction in pain
Raeissadat et al. [27] BMC Sports Sci Med and Rehab 2014	ACD-A (2ml)	4.8×	Not mentioned	No	Lidocaine	1600RPM - 15min /2800RPM - 7min	No	No	2 ml	After 12 months, 75% of subjects had a reduction in pain by >25%
Tonk et al. [28] Indian J of Ortho 2014	ACD-A (3 mg)	5×	Not mentioned	No	1 ml of 2% xylocaine	700 RPM–20 min/ 1750 RPM–15 min	No	No	3 ml	Reduction in Nirschl score for both groups Better results in the short term for laser and long term for PRP
Chiavaras et al. [29] Acad Radiol 2014	No	Not mentioned	Not mentioned	No	1% lidocaine	1500 RPM–5 min	No	Yes	3 ml	Forthcoming
Ford et al. [30] American Association for Hand Surgery 2014	ACD-A (1 ml)	Not mentioned	Not mentioned	Not mentioned	5 ml of 1% lidocaine	1500 RPM–5 min	Not mentioned	No	3–4 ml	Reduction in pain for 89.3%

Table III. Various clinical studies using platelet rich plasma to treat Achilles tendinopathies.

Study	Anticoagulent used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	PRP volume injected	Platelet2 concentration	Results
Kon et al. [31]INT J Care Injured 2009	Sodium Citrate (21ml)	6×	Not mentioned	Calcium chloride (10%)	No	1800 RPM–15 min/ 3500RPM–10 min	Not mentioned	No	3 injections of 5 ml of PRP (1 every 15 days)	6×	Improved VAS, Tegner, SF-36 80% of patients satisfied At 6 months, 70%of patient's symptoms had reduced disappeared. Without physical therapy, the results were less good (Tegner and EQ-VAS)
Filardo et al. [32] International Orthopedics 2010	Not mentioned	6×	Not mentioned	Calcium chloride (10%)	No	1800RPM - 15 min/ 3500RPM–10 min	Not mentioned	No	3 injections of 5 ml of PRP (1 every 15 days)	6×	Reduction in VAS for both groups Improved Tegner score better for the PRP group (29% vs. 20%)
Brown et al. [33] PM&R 2010	ACD-A	Not mentioned	Not mentioned	No	1% lidocaine	3200 RPM - 15 min	Not mentioned	Yes	3 ml	Not mentioned	Improved by 19 points for VISA-P score, 50% reduction in pain. Under ultrasound, reduction in tendon thickness and hypoechogenicity, At 8 months, patient satisfied.
Scollon-Grieve et al. [34] PM&R 2011	Not mentioned	Not mentioned	Not mentioned	Not mentioned	3 ml of lidocaine 1%	Not mentioned	Not mentioned	Yes	5 ml	Not mentioned	At 1 month 90% clinical and functional improvement and disappearance of pain. Improved echogenicity under ultrasound, at 2 months, complete activity without pain or limitation.
Gosens et al. [35] International Orthopedics 2012	ACD-A	Not mentioned	Not mentioned	No	Bupivacaine, epinephrine	3200 RPM–15 min	8.4% sodium bicarbonate	No	1 ml	Not mentioned	Significant improvement for both groups for VAS, larger for the VISA-P for the group only with PRP
Ferrero et al. [36] Journal of Ultrasound 2012	Not mentioned	Not mentioned	Not mentioned	Not mentioned	4ml of 2% mepivacaine	Not mentioned	Not mentioned	Yes	2 injections of 6 ml of PRP three weeks apart	Not mentioned	No improvement at 20 days but significant at 6 months of VISA, at 6 months reduction in hypoechogenicity and tendon thickness, intra-tendenous vascularization increased at 20 days and 6 months.

(*Continued*)

Table III. (Continued).

Study	Anticoagulent used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	PRP volume injected	Platelet2 concentration	Results
Vetrano et al. [37] Am J Sports Med 2013	ACD-A	3 to 5×	Not mentioned	No	No	1500 g - 10 min	Not mentioned	Yes	3–5 ml	3.5×	After 12 months, 91% of patients were satisfied
Filardo et al. [38] International Orthopedics 2013	Not mentioned	Not mentioned	Not mentioned	Calcium chloride	No	1480 RPM–6 min/ 3400 RPM–15 min	Not mentioned	Yes	3 × 5 ml at two week intervals	Not mentioned	Improvement over time (VISA-P, EQUAS, Tegner). No results for very old symptoms and bilateral lesions.
Van Arket al. (3ç) Physical Therapy in Sport 2013	ACD-A	Not mentioned	Not mentioned	Not mentioned	Not mentioned	3500 RPM–5 min	Not mentioned	Yes	Not mentioned	Not mentioned	1 result with improvement lower than 30 points for VISA-P at 26 weeks
Dragoo et al. [40] Am J Sports Med 2014	ACD-A	Not mentioned	Not mentioned	Not mentioned	Bupivacaine, epinephrine	3200 RPM–15 min	Not mentioned	Yes	6 ml	Not mentioned	Better VISA-P results at 12 weeks for the PRP group but greater significant difference after 26 weeks between the two groups. No difference in evolution between the 2 groups for the Tegner, VAS and SF-12 scores.
Dallaudière et al. [41] J Vasc Interv Radiol 2014	ACD-A	3×	200 leukocytes/ mm^3	Not mentioned	10 ml of lidocaine 1%	620 g–15 min	Not mentioned	Yes	3 ml	3×	Significant improvement in WOMAC score (16.1 to 6) Reduction in lesions under ultrasound
Charousset et al. [42] Am J Sports Med 2014	ACD-A	2×	Not mentioned	No	No	1700 RPM – 5 min	Not mentioned	Yes	3× 2 ml per week	2×	Improved scores (VISA-P, VAS, Lysholm) after 2 years 21 patients regained their sporting level at 3 months. 3 underwent surgery. At three months, 57% normal structure of the tendon under ultrasound.
Smith et al. [43]Clin J Sport Med 2014	Not mentioned	Not mentioned	Not mentioned	No	No	Not mentioned	Not mentioned	Yes	2× 2 ml per week	Not mentioned	At 12 months, improved VISA-P and VAS scores. Satisfaction 91.3%

(*Continued*)

Table III. (Continued).

Study	Anticoagulent used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	PRP volume injected	Platelet2 concentration	Results
Kaux et al. [44] J Sports Med Phys Fitness 2014	ACD-A	900,000 platelets/µL	Nearly no erythrocytes or leukocytes	Calcium chloride (300 µL)	No	Not mentioned (apheresis machine)	Not mentioned	Yes	6 ml	900,000 platelets/µL	Improved VAS, IKDC, VISA-P scores and the pressure algometer at 3 months No significant difference under ultrasound, increase in tendon thickness and sagittal hypersignal under MRI
Kaux et al. [45] Acta Orthopedic Belgica 2015	ACD-A	900,000 platelets/µL	Nearly no erythrocytes or leukocytes	Calcium chloride (300 µL)	No	Not mentioned (apheresis machine)	Not mentioned	Yes	6 ml	900,000 platelets/µL	71% of patients showed a favorable evolution with a reduction in pain and return to sporting activities. Significant improvement in VAS, IKDC, VSA-P.
Kaux et al. [46] J Sciences & Med in Sports 2015	Not mentioned	900,000 platelets/µL	Nearly no erythrocytes or leukocytes	Calcium chloride (300 µL)	No	Not mentioned (apheresis machine)	Not mentioned	Yes	6 ml	900,000 platelets/µL	No significant difference between the two groups (one injection vs. two injections)
Zayni et al. [47] Muscles, Ligaments and Tendons Journal 2015	ACD-A	2×	No leukocytes	No	Not mentioned	1700 RPM - 5 minutes	Not mentioned	Yes	6 ml	2×	After 34 months of follow-up, patients who had received 2 injections evolved better than those who received 1 injection, 86% of patients were able to resume their level of activity.

Table IV. Various clinical studies using platelet rich plasma to treat plantar fasciitis.

Study	Anticoagulent used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	PRP volume injected	Results
De Vos et al. [48] JAMA 2010	Citrate	Not mentioned	Not mentioned	Not mentioned	0.5% marcain	Not mentioned	8.4% sodium bicarbonate	Yes	4 ml	Increase in VISA-A for two groups (PRP vs. saline solution) without any significant difference.
Gaweda et al. [49] Int J Sport Med 2010	Citrate Phosphate Dextrose Adenine (CPDA)	Not mentioned	Not mentioned	Not mentioned	Not mentioned	2400 RPM–10 min/3600 RPM - 15 min	Not mentioned	Yes	3 ml	Significant improvement at 6 weeks, 3, 6 and 18 months, AOFAS and VISA-A scores. Ultrasound showed reduction in tendon thickness but vascularization still present.
De Jonge et A. [50] Am J Sports Med 2011	Sodium citrate	Not mentioned	Not mentioned	No	0.5% marcain	Not mentioned	8.4% sodium bicarbonate	Yes	4 ml	Improved VISA-A for the two groups without any significant difference. Satisfaction at 59.3% for each group. Return to same sporting level 56.5% PRP and 41.7% saline injection. No difference between the groups under ultrasound.
Owens et al. [51] Foot Ankle Int 2011	Not mentioned	4 to 6 x	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Yes	6 ml	Improvement in SF-8, FAAM and FAAMS scores. For the six patients who had an MRI pre and post-injection, reduction in the length of the damaged segment, but not significant.
Monto. [52] Foot Ankle Int 2012	ACD-A	Not mentioned	Not mentioned	No	0.5% ropivicaine	2400 RPM - 12 minutes	Not mentioned	Yes	4 cc	Improved AOFA®€ MRI imaging and/or ultrasound shows tendon healing at 24 months. Final assessment, 28 patients out of 30 satisfied.
Deans et al. [53] J Foot Ank Surg 2012	ACD-A	Not mentioned	Not mentioned	Not mentioned	No	1500 RPM - 5 min	Not mentioned	No	3 ml	Significant improvement in pain, daily and sporting life, quality of life.
Ferrero et al. [36] Journal of Ultrasound 2012	Not mentioned	Not mentioned	Not mentioned	Yes (not mentioned)	2% mepivacaine	Not mentioned	Not mentioned	Yes	6 ml	VISA-A improved at 6 months. Lower hypoechogenicity tendon thickness. Increased intratendenous vascularization throughout.
Mautner et al. [54] PM&R 2013	Not mentioned	Not mentioned	Not mentioned	No	Not mentioned	Not mentioned	Not mentioned	Yes	Not mentioned	96% of patients' symptoms completely disappeared.
Kearney et al. [55] Bone Joint Res 2013	Sodium citrate	Not mentioned	Not mentioned	Not mentioned	Not mentioned	2400 RPM - 12 minutes	Not mentioned	No	3–5 ml	Improved VISA-A, EQ-5D and VAS scores for both groups but no significant difference (PRP vs. excentrique physical therapy)

(*Continued*)

Table IV. (Continued).

Study	Anticoagulent used	Platelet concentration	Presence of white and red blood cells	Platelet activators	Local anesthetic	Centrifugations	pH buffer	Ultrasound guidance	PRP volume injected	Results
Murawski et al. [56] Foot Ankle Spec 2014	ACD-A	Not mentioned	Not mentioned	Not mentioned	0.1% lidocaine	1200g - 17min	Not mentioned	No	3 ml	At six months, 78% of patients were asymptomatic, improved FAOS and SF-12 scores. 22% failure and surgery required. Overall, no difference under MRI.
Dallaudière et al. [41] Jvasc Interv Radiol 2014	ACD-A	3×	200 leukocytes/mm^3	Not mentioned	10 ml of lidocaine 1%	620 g–15 min	Not mentioned	Yes	3 ml	Significant improvement in WOMAC and VAS at 6 weeks and 32 months. Reduction in lesions under ultrasound
Filardo et al. [57] Blood Transfus 2014	Not mentioned	5×	Presence of leukocytes (1.2×)	Calcium chloride	Yes (not mentioned)	1480 RPM–6 min/3600 RPM–15 min	Not mentioned	Yes	3 × 5 ml at two week intervals	Improvement in VISA-A and EQ-VAS scores up to six months and now after 4.5 years. Tegner score continuously improving.
Oloff et al. [58] Sports Orthop Rehab Med Associates 2015	ACD-A	2×	Not mentioned	No	Not mentioned	3200 RPM–15 min	8.4% sodium bicarbonate	No	Not mentioned	Improved VISA-A. No significant difference between the two groups (PRP injection vs. injection of PRP during surgery).

terms of the type of anticoagulant, most authors agree on the nonuse of EDTA (ethylene diamine tetra-acetic acid) which may damage the platelet membrane. Hence, we will focus on the use of anticoagulants containing citrate-dextrose or sodium citrate [70].

Acid-citrate-dextrose (ACD) is an anticoagulant used by blood banks to store viable platelets for transfusion. ACD can maintain platelet viability for six hours [71]. In a study analyzing the effects of anticoagulants on PRP quality, Lei et al. [72] highlighted that ACD maintains the integrity of the structure of platelets in the PRP for up to 12 hours. However, when heparin sodium and sodium citrate are used, half of the platelets begin to rupture in the first hours. The positive effect of ACD on maintaining platelet viability may be due to glucose and the low citrate concentration [73]. One alternative to ACD-A is citrate phosphate dextrose-adenine (CPDA). This anticoagulant is similar, but is 10% less effective in terms of maintaining platelet viability [74].

Centrifugation speed

Several factors may influence the integrity of platelets as well as the composition and effectiveness of PRP. These include the number of revolutions per minute (RPM), centrifugal acceleration and duration of centrifugation, and angular speed and distance separating the platelets from the rotor axis.

Perez et al. [75] attempted to highlight elements of the centrifugation stage required to obtain reproducible and high-quality results. Blood samples were taken from 20 healthy donors. Two stages of centrifugation were analyzed to identify the influence of centrifugal acceleration, duration, the volume treated, and the platelet gradient. They concluded that low centrifugal promoted the separation of PRP. Treating 3.5 ml of blood at 100g for 10 minutes (first centrifugation) followed by a second centrifugation at 400g for 10 minutes, which had previously removed 2/3 of the remaining plasma, enabled a higher rate (70–80%) and higher concentration (5x) of platelets to be recovered while maintaining platelet integrity and viability. When using a larger volume (8.5 ml), platelet recovery was lower. The authors therefore concluded that centrifugal acceleration, the duration of centrifugation, the platelet gradient prior to sampling, and the volume treated were the main elements to be retained in order to ensure a reproducible composition of PRP.

In contrast to the study by Perez et al. [75], a recent study by Arora et al. [76] showed better results with centrifugal acceleration of 440g for 10 minutes. This difference may be due to the difference in the initial volume used for the preparation of PRP. There are numerous protocols in the current literature which describe the optimal conditions for centrifugation.

Platelet concentration

To date, very few studies have been carried out on the optimal dosage of platelets in PRP. Numerous researchers have, however, suggested that platelet concentration must be three to seven times higher than that found in peripheral blood (between 150,000 and 350,000 platelets/μL) [77,78]. Weibrich et al. [79] assessed, in vivo, the effects of different platelet concentrations on bone regeneration. He found that the use of platelet concentrate had positive effects, on the condition that the platelet concentration was approximately between 503,000 and 1,729,000 platelets/μL (1.5 to 4.5 times the concentration of platelets in peripheral blood). Indeed, the use of an excessively high platelet rate (6 to 12 times the concentration of platelets in peripheral blood) appeared to have an inhibitory effect on healing. Graziani et al. [78] assessed the effect of different concentrations of PRP on osteoblast and fibroblast function in vitro. The results of this study showed that PRP has a maximum effect when obtained with a platelet concentration which is 2.5 times higher than the concentration in peripheral blood. Increased concentrations lead to a reduction in the proliferation of osteoblasts and fibroblasts, suggesting a paradoxical inhibitory effect on tissue regeneration.

The large variety of platelet concentrations encountered in many studies is explained by the use of commercial kits to prepare PRP. The results of these kits vary significantly from one patient to another but also from one sample to another for the same patient [13,14]. Only use of an apheresis machine can obtain a constant concentration and reproducibility for each patient [80]. The donation of blood components through apheresis has become commonplace in modern blood transfusion practices. Technological progress in automated cell separators has improved the productivity and quality of platelet collection through apheresis [81]. There are a wide variety of instruments to extract platelets through apheresis, and many studies have compared different cell separators. Of those, Keklik et al. [82] compared three apheresis systems (Fenwal Amicus, Fresenius COM.TEC and Trima Accel) in terms of processing time, platelet output, efficiency, and speed of collection. The results showed that the volume of blood treated, the volume of ACD-A, and the average separation time were significantly higher with the COM.TEC system.

Altuntas et al. [83] compared two apheresis systems (Fenwal Amicus and Fresenius COM.TEC), comparing processing time, platelet output, efficiency of collection, and white cell content. The results obtained showed that both instruments collected platelets efficiently with an equal leukocyte content; however, Amicus achieved the desired platelet rate more quickly.

Moog et al. [80] demonstrated that the COM.TEC machine enabled platelet concentrates to be acquired with a lower rate of leukocytes meeting the standards in place. To do so, five centers collected 554 samples using the COM.TEC cell separator. Two cell counting studies were carried out at the start and at the end of the study to confirm a uniform count between the participating centers.

The impact of the concentration of red and while blood cells on PRP actions

The presence of red and white blood cells in the PRP may also impact upon the effectiveness of the treatment.

Indeed, the lysis of erythrocytes can cause the generation of highly reactive oxygen metabolites (also called oxygen-deviated free radicals) which can cause tissue damages and may hinder the healing process [84].

Moreover, the release of pro-inflammatory factors (cytokine and metalloproteinase) by the leukocytes may lead to deterioration of the extracellular matrix. Zhang et al. [85] published the hypothesis that PRP containing high levels of leukocytes (L-PRP) increases the concentration of catabolic cytokine and induces inflammation and apoptosis of tendon cells. This study compared the effects of L-PRP and PRP on the morphology, proliferation, and differentiation of tendon cells. The results of this study showed that L-PRP has negative effects on tendon stem cells by inhibiting their proliferation, accelerating their differentiation and inducing their apoptosis. McCarrel et al. [86] also came to the conclusion that a low concentration of leukocytes leads to a reduction in the inflammatory expression of cytokines. Another study carried out on patellar tendons in white mice by Dragoo et al. [87] compared the inflammatory effects of an injection of PRP containing high levels of leukocytes (L-PRP) to those of an injection of PRP with few leukocytes. The results showed that the L-PRP provoked a significant inflammatory response for five days after the injection. The authors observed an increased rate of

leukocytes and mononuclear cells (macrophages and lymphocytes) in the tendons treated with L-PRP in comparison to those treated with PRP. They also noted an increase in vascularization and fibrosis of these tissues.

Pizza et al. [88] studied the effects of neutrophiles (pro-inflammatory factors) on the extracellular muscle matrix. It appeared that the neutrophiles could damage the skeletal myotubes in vitro and aggravate injuries or delay tissue regeneration in vivo.

With these observations, we recommend the use of leukocyte poor PRP (and without any erythrocytes) to avoid any local inflammatory reaction which can be painful for the patient and reduce the proliferative phase of the healing process. However, in the clinical literature in human, the results can also be good with the use of L-PRP; the disparity of the PRP used would be responsible for the variability of the results obtained. Thus, a general agreement on the preparation and the type of the PRP to use in orthopedics is still need.

Platelet activator

Activation of PRP prior to the injection is another parameter which requires in-depth examination. Platelet activation leads to the degranulation of alpha granules. The release of numerous proteins and growth factors (including PDGF, TGF-β, VEGF, and IGF) stimulates the healing process [10,89]. Platelets may be activated exogenously by thrombin, calcium chloride, or as the result of mechanical trauma. Denatured collagen is a natural PRP activator, and when used within soft tissue, exogenous activation is not always necessary [90,91].

Once the PRP is activated, a fibrin network starts to form, solidifying plasma and creating a fibrin clot or membrane which contributes toward tissue healing. According to Weibrich et al. [92], no significant changes in platelet or growth factor concentration are observed according on the patient's age or sex. Several studies, however, report that the hematocrit and total platelet level influence the platelet concentration of PRP [92,93]. Indeed, the higher the hematocrit and platelet levels are, the greater the platelet concentration will be.

A study by Martineau et al. [94] showed that PRP activation with a high concentration of calcium and thrombin leads to an immediate and significant increase in the release of growth factors.

Local anesthetic

Although intratendon injections are painful, the use of a local anesthetic is not recommended as it may compromise the therapeutic potential of PRP: The anesthetic may reduce local pH, leading to an inhibition, reduction, or absence of platelet degranulation [16]. This hypothesis has been confirmed by Bausset et al. [95] who showed that the association formed by a local anesthetic (lidocaine, ropivacaine) and PRP injections in vitro leads to a significant reduction in platelet functionality, in particular platelet aggregation. He therefore recommends using smaller needles. Carofino et al. [96] tried to identify the effects of the association formed by an anesthetic (lidocaine, bupivacaine) or a corticosteroid (methylprednisolone) and PRP on human tenocytes in vitro. He came to the conclusion that adding one or the other to the PRP resulted in a significant reduction in tenocyte proliferation and cell viability.

Ultrasound guidance

Many studies do not use ultrasound guidance. In such cases, the area to be treated is identified by palpation, followed by a series of injections in order to obtain complete coverage of the site [97]. The growing use of ultrasound to guide injections enables the PRP injection into the pathological tendon to be visualized. The basic principle of PRP is to inject a high concentration of active proteins and growth factors to the injury site. It would, therefore, appear relevant to identify the extent to which the product effectively remains at the injection site and what quantity is dispersed into the surrounding tissue. It has, nonetheless, been demonstrated that PRP diffused several centimeters from the injection site in the minutes following infiltration [97].

Discussion

Although there is an increasing amount of scientific proof about the benefits of PRP infiltration in the treatment of tendinopathies, there is currently no consensus as to how to obtain or use it. This may partly explain the disparity of results obtained in the literature. Similarly, the biological parameters and risk factors for tendinopathies of patients themselves may possibly influence the results of this treatment even if, to date, this has not yet been demonstrated [98].

Analysis of these studies (Tables I–IV) shows that a majority use ACD-A as an anticoagulant during sampling. By comparing the results of studies using ACD-A and sodium citrate (seven studies), we were able to highlight the greater effectiveness of ACD-A. However, the small number of studies using sodium citrate does not allow us to confirm this hypothesis.

A platelet activator is only used in a few studies (eight studies). Of those, calcium chloride is the main product used. Each study using this platelet activator presents positive results. However, the best results have been obtained in studies which use no platelet activator.

It is, however, important to note that the results are obtained by adding a range of variables which are not reproducible from one study to the next (anticoagulant, platelet activator, local anesthetic, speed of centrifugation, pH buffer, ultrasound guidance, volume of PRP injected). As such, it is impossible to draw conclusions about the greater effectiveness of ACD-A and the real value of using a platelet activator.

In terms of the speed of centrifugation, analysis of all these studies appears to confirm the existence of a great number of protocols in the literature. It is difficult to draw any conclusions when these different variables are unknown (volume of initial sample, equipment used to obtain the PRP).

Currently, no studies have been carried out neither on the use of a "buffer" product to neutralize the acidity caused by the anticoagulant nor on the optimal volume of PRP to be injected.

Although it would appear inadvisable to administer a local anesthetic because the anesthetic may reduce the local pH and lead to the inhibition, reduction, or absence of platelet degranulation, the literature shows that the majority use a local anesthetic (32 studies out of 57). They do not, however, obtain better results than those not using a local anesthetic.

Finally, it appears to be advisable to carry out infiltration under ultrasound guidance, even if PRP diffusion is observed after injection.

This all aims to show that there is still a need for high quality studies, with standardized collection protocols and the use of PRP in the context of tendinopathies, in order to better scientifically understand the real effectiveness.

Declaration of interest

The authors report no conflicts of interest

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