Platelet-rich plasma in the treatment of equine orthopaedic disease

Platelet-rich plasma is a blood-derived, autologous product, which contains a mixture of growth factors, cells and cytokines. These substances are integral in the regulation of the inflammatory process and repair of tissues, although their methods of action are highly complex and not fully elucidated. The content of a platelet-rich plasma product is variable and the optimal concentrations of prime constituents such as platelets, growth factors and leucocytes are not known. A lack of uniformity of products and treatment protocols, along with study design limitations, means that the efficacy of platelet-rich plasma in healing tendon and ligament injuries is yet to be proven or disproven. Nevertheless platelet-rich plasma has gained widespread use in clinical practice primarily for the treatment of these injuries, among other applications. There are no widespread published or anecdotal concerns over the safety of platelet-rich plasma; however, synovial fluid analysis reveals an acute inflammatory response following intra-articular injection of a leucocyte-rich product.

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Rachel Tucker BSc, BVetMed, MVetMed, CertAVP(ESO), Dip.ECVS, MRCVS, RCVS and European specialist in equine surgery, Liphook Equine Hospital, Forest Mere, Liphook, Hampshire GU30 7JG. rachel.tucker@theleh.co.uk

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Platelet-rich plasma (PRP) is a cell-based therapy, and is a popular therapeutic option for the treatment of a number of musculoskeletal conditions in horses. PRP is defined as a blood-derived product that contains an increased concentration of platelets compared to that of peripheral blood. This article reviews the production, composition and possible mechanism of action of PRP, with the aim of helping to make decisions regarding its use in clinical practice. Evidence of efficacy will be summarised, highlighting the challenges of evaluating the clinical effect of biologic products.

What does PRP do?

Platelets consist of alpha granules, which contain a multitude of growth factors and cytokines that are released at sites of vascular injury to direct and promote healing (Pochini et al, 2016). Growth factors such as transforming growth factor β1 (TGF-β1), insulin-like growth factor 1 (IGF-1), and platelet-derived growth factor (PDGF) instruct specific cellular responses. For example, TGF-β1 and IGF-1 increase synthesis of extracellular matrix, while PDGF promotes angiogenesis. IGF-1 has also been shown to decrease synovial inflammation. Cytokines are smaller proteins that regulate communication between cells. Cytokines such as tumour necrosis factor and interleukins 6, 8 and 10 are fundamental in the regulation of inflammation and the subsequent formation of a tissue scaffold that enables formation of new extracellular matrix in injured tendon (Gaida et al, 2012). In addition, platelet activation releases chemokines that recruit leucocytes to a site of injury (King et al, 2018). Collecting and concentrating platelets and delivering them directly to the site of musculoskeletal injury aims to harness these effects to improve the speed and quality of tissue healing, as well as providing anti-inflammatory effects.

While the use of growth factors and cytokines within PRP has a solid rationale, the complexity of their actions makes manipulating and measuring their effects very challenging. Overall, these molecules influence the behaviour of different tissues and of other bioactive factors in a variety of ways and are capable of having pro- and anti-inflammatory effects (Zhang and An, 2007). Many cytokines can act within opposing biological pathways and while some growth factors are beneficial in certain applications, the same growth factors are deleterious in others (Wahl et al, 1989). Overall, the effect of PRP is likely to depend on the relative composition of the product, the local environment into which it is delivered and the stage and degree of injury present.

PRP preparation

PRP is an autologous product created by either centrifugation or filtration of a sample of the patient's blood. This yields a platelet-rich fraction, which is then separated and delivered to the site of disease or injury by injection. Filtration can be performed 'patient side', allowing PRP preparation and injection during the same consultation

(*Figure 1*). Alternatively, the blood sample may be spun in a centrifuge and separated to yield the platelet-rich fraction. Centrifugation time, spin rate (revolutions per minute) and separation method depends on the specific commercial system used. Individual instructions should be followed for each product and use of an appropriately sized centrifuge is required. Strict asepsis must be maintained at each step of the process to prevent iatrogenic infection, which could have disastrous consequences. Products that use a closed system reduce the risk of bacterial contamination of the PRP dose.

PRP composition

The composition of PRP varies between products, method of preparation and between patients, with the most studied variables being platelet, growth factor and leucocyte concentrations (Fitzpatrick et al, 2017a). Platelet concentration is not directly proportional to the biologic potential of the product, as platelets must degranulate to release their active components. Measuring growth factor concentrations is helpful, but further platelet degranulation will occur at the site of treatment, for example by exposure to collagen I fibres (Bonilla-Gutiérrez et al, 2019). A comparison of these values measured in a number of commercially available products is shown in *Table 1* (Hessel et al, 2015).

Patient factors at the time of blood sampling are also known to affect PRP composition. For example, platelet concentrations were increased in horses that had received previous intravenous non-steroidal anti-inflammatory drug therapy, whereas leukocyte concentration was increased by patient dehydration and blood sampling at night (Rinnovati et al, 2015).

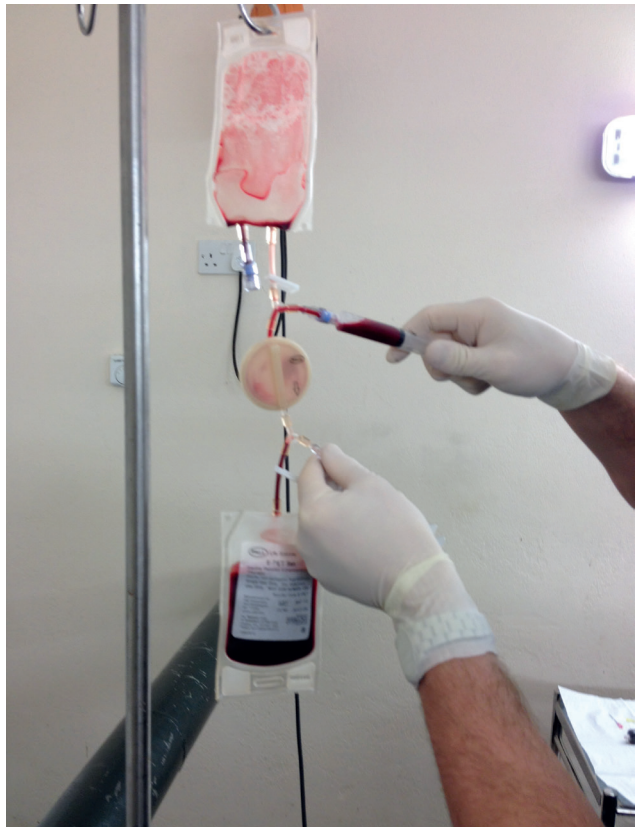

Figure 1. 'Patient side' preparation of platelet-rich plasma using a filtration system. PRP can be seen in the top syringe.

Table 1. Composition of a sample of commercial platelet-rich plasma products to demonstrate product variability

Variable	Concentration	Baseline blood	PRP product (manufacturer)			
			Angel (Arthrex)	ACP (Arthrex)	GPS (Biomet Biologics)	E-PET (PALL Corporation)
Platelets	Mean (10^9/litre)	140.3	320.3	183.2	761	533.3
	Standard deviation	28.4	198.1	39.7	240	198.2
	Mean enrichment		1.80x	1.30x	5.27x	3.69x
White blood cells	Mean (10^9/litre)	6.1	9.1	0.6	40.6	11
	Standard deviation	0.5	6	0.3	3.9	2.5
	Mean enrichment		1.16x	0.09x	6.63x	1.76x
Platelet-derived growth factor	Mean (ng/ml)	0.44	2.44	0.85	5.16	5.27
	Standard deviation	0.19	0.69	0.05	1.12	1.61
	Mean enrichment		5.14x	1.84x	12.51x	12.55x
Transforming growth factor β1	Mean (ng/ml)	0.30	0.66	0.28	0.68	1.70
	Standard deviation	0.05	0.06	0.09	0.29	0.41
	Mean enrichment		2.23x	0.93	2.17x	5.60x

Adapted from Hessel et al, 2015

In general, all products increase the concentration of platelets and growth factors but they variably concentrate or dilute the level of white cells (leucocytes) (*Table 1*), resulting in a leucocyte rich (LR-PRP) or leucocyte poor (LP-PRP) preparation. Leucocyte levels are relevant as they can recruit and alter the effects of growth factors and cytokines at a local level, as well as having their own effects (Sundman et al, 2011). Leucocytes have been shown to be inflammatory within a synovial environment, significantly elevating total white cells, neutrophils and total protein within healthy and diseased equine joints, following administration of LR-PRP (Textor and Tablin, 2013; Smit et al, 2019). Although generally considered an undesirable effect, the significance of this inflammatory response is debated and suggested to be self-limiting (Moraes et al, 2015; King et al, 2018). In a meta-analysis of 18 randomised controlled human trials, highly cellular LR-PRP was more beneficial in improving pain and function scores than LP-PRP in the treatment of tendinopathy (Fitzpatrick et al, 2017b), but an explanation for this difference was not discussed.

The optimum platelet and leucocyte concentrations in PRP are not known and are likely to vary between joint and soft tissue applications. Boswell et al (2014) found that increasing platelet numbers above a certain threshold resulted in decreased collagen synthesis in superficial digital flexor tendon explants, indicating that more is not necessarily better when it comes to platelet levels.

PRP administration

PRP is most commonly administered as an intralesional injection into ligaments and tendons. The aim is to fill the lesion, which is viewed as a hypoechoic region on an ultrasound image (*Figure 2*). Core defects, or those with an intact tissue border are most suitable, in order to retain the PRP at the site of injection. Injection is usually performed in the standing sedated horse, with local anaesthesia provided by local infiltration or regional perineural anaesthesia. A high 4-point nerve block is appropriate for most injections of the palmar metacarpus/metatarsus. The site is identified ultrasonographically and the injection is performed under direct ultrasound guidance, using an aseptic technique. It is helpful to have an assistant who is able to adjust the scanner and pass items to the treating clinician, who can simultaneously scan the site and administer the injection. Common injection sites include the suspensory ligament (origin, body and branch), accessory ligament of the deep digital flexor tendon (inferior check ligament), superficial digital flexor tendon and collateral ligaments (e.g. distal interphalangeal joint). PRP is also used by some clinicians as an intra-articular or intrathecal injection for the management of osteoarthritis, synovitis and soft tissue injuries, particularly when corticosteroids are contraindicated. In this application, it may seem logical to use a leucocyte-poor product with no red cell component to minimise any inflammatory response; however, this rationale is debated (King et al, 2018).

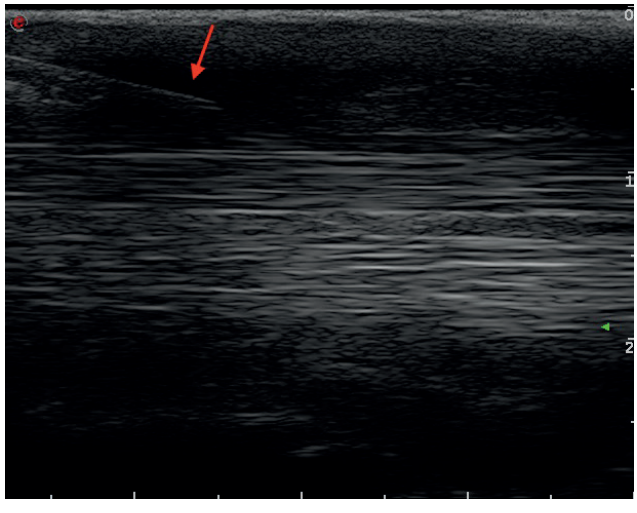

Figure 2. M-mode 12MHz longitudinal ultrasound image of the palmar metacarpus during injection of the superficial digital flexor tendon (SDFT). The tip of a 21G needle is evident within a marginal hypoechoic SDFT defect before intralesional injection (arrow).

KEY POINTS

- Platelet-rich plasma (PRP) is created by either centrifugation or filtration of a sample of the patient's blood to yield a platelet-rich product which is delivered to a site of musculoskeletal disease or injury by injection.
- PRP is a source of growth factors, cytokines and other bioactive substances which play important roles in the regulation of tissue repair and inflammation.
- Common sites of injection are lesions of the suspensory ligament, check ligaments, flexor tendons and collateral ligaments.
- Although not quantified, intralesional injection into soft tissue structures is considered a low risk procedure so long as aseptic conditions are maintained throughout.
- The variability of the PRP product and the complexity of its actions hinders our ability to assess its efficacy.

Does PRP work?

In vitro, PRP has been shown to have anabolic effects on tendon and ligament explants: increasing gene expression of collagen I and III, increasing DNA content (cellularity) and influencing fibroblast proliferation (Smith et al, 2006; Schnabel et al, 2008). Note that healthy tendon is relatively acellular, hence increasing cellularity is not necessarily synonymous with optimal repair. Intralesional administration of PRP 1 week after experimental induction of superficial digital flexor tendon core lesions resulted in significantly increased DNA, collagen and glycosaminoglycan content at 6 months post-lesion creation, compared with controls. Increased tensile strength and elastic modulus was also noted, suggesting a stronger but stiffer tendon following repair (Bosch et al, 2009).

Despite numerous other studies also showing the beneficial effect of PRP in the healing of tendons and ligaments, definitive clinical evidence of its efficacy is still lacking. This is because the majority of equine clinical studies that yield positive results also demonstrate sufficient positive bias to raise questions over their findings. The most frequent flaws in clinical trials are a lack of a true placebo group, poor product characterisation, insufficient blinding, small sample size, short follow-up period and adoption of poor outcome measures

(Brossi et al, 2015; Bonilla-Gutiérrez et al, 2019). In humans, a meta-analysis of 18 clinical trials (1066 participants) concluded that there is evidence to support a single ultrasound guided injection of LR-PRP in the treatment of tendinopathy (Fitzpatrick et al, 2017a). A systematic review of five trials (214 participants) into the treatment of rotator cuff disease concluded that PRP did not result in superior functional outcomes, pain scores or range of motion when directly compared with exercise therapy, but may have a role in the promotion of tendon healing (Hurley et al, 2019).

With regard to intra-articular use, a review of the basic science evidence of the role of PRP in cartilage pathology reviewed 27 studies and summarised that PRP decreased inflammation in 75% of the in vitro studies and resulted in improved histological quality of the cartilage tissue in 75% of the in vivo studies reporting data. This review was limited by poor quality reporting of methodology and PRP content in the included studies (Fice et al, 2019). A meta-analysis of 34 human studies (1403 participants) reported that Western Ontario and McMaster Universities Osteoarthritis Index scores (assessing pain, stiffness and function) significantly favoured the use of PRP over placebo or hyaluronic acid at 12-month follow up, with lesser or no benefit at earlier time points. The authors concluded that PRP should be regarded as having a longer lasting effect rather than producing greater improvement (Filardo et al, 2020). As yet, there is insufficient clinical evidence to usefully evaluate the effect of PRP in pathological equine joints (Mirza et al, 2016; Tyrnenopoulou et al, 2016).

Conclusion

PRP offers an attractive treatment option as it is autologous, can be prepared patient side, is relatively easy to administer, low risk and relatively affordable compared with some other options. However, as with many equine orthopaedic treatments, an evidence-based rationale for its use is still lacking. In these instances, some understanding of the underlying cell biology of the product and what is currently known about its effects is important when deciding whether and how to use the product. EQ

References

Bonilla-Gutiérrez AF, López C, Carmona JU. Regenerative therapies for the treatment of tenodesmic injuries in horses. J Equine Vet Sci. 2019;73:139–147. https://doi.org/10.1016/j.jevs.2018.12.010

Bosch G, van Schie HTM, de Groot MW et al. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: a placebo-controlled experimental study. J Orthop Res 2009;28(2):211–217. https://doi.org/10.1002/jor.20980

Boswell SG, Schnabel LV, Mohammed HO, Sundman EA, Minas T, Fortier LA. Increasing platelet concentrations in leukocyte-reduced platelet-rich plasma decrease collagen gene synthesis in tendons. Am J Sports Med. 2014; 42(1):42–9. https://doi.org/10.1177/0363546513507566

Brossi PM, Moreira JJ, Machado TS, Baccarin RY. Platelet-rich plasma in orthopedic therapy: a comparative systematic review of clinical and experimental data in equine and human musculoskeletal lesions. BMC Vet Res. 2015;11(1):98. https://doi.org/10.1186/s12917-015-0403-z

Fice MP, Miller JC, Christian R, Hannon CP, Smyth N, Murawski CD, Cole BJ, Kennedy JG. The role of platelet-rich plasma in cartilage pathology: an updated systematic review of the basic science evidence. Arthrosc J Arthrosc Relat Surg. 2019;35(3):961-976.e3. https://doi.org/10.1016/j.arthro.2018.10.125

Filardo G, Previtali D, Napoli F et al. PRP Injections for the treatment of knee osteoarthritis: a meta-analysis of randomized controlled trials. Cartilage. 2020. https://doi.org/10.1177/1947603520931170

Fitzpatrick J, Bulsara M, Zheng MH. The effectiveness of platelet-rich plasma in the treatment of tendinopathy: a meta-analysis of randomized controlled clinical trials. Am J Sports Med. 2017a;45(1):226–233. https://doi.org/10.1177/0363546516643716

Fitzpatrick J, Bulsara MK, McCrory PR, Richardson MD, Zheng MH. Analysis of platelet-rich plasma extraction: variations in platelet and blood components between 4 common commercial kits. Orthop J Sports Med. 2017b;5(1):232596711667527. https://doi.org/10.1177/2325967116675272

Gaida JE, Bagge J, Purdam C et al. Evidence of the TNF-α system in the human achilles tendon: expression of TNF-α and TNF receptor at both protein and mRNA levels in the tenocytes. Cells Tissues Organs. 2012;196:339–352. https://doi.org/10.1159/000335475

Hessel LN, Bosch G, van Weeren PR, Ionita J-C. Equine autologous platelet concentrates: A comparative study between different available systems: A comparison of five autologous platelet concentrate products. Equine Vet J. 2015;47(3):319–325. https://doi.org/10.1111/evj.12288.

Hurley ET, Hannon CP, Pauzenberger L et al. Nonoperative treatment of rotator cuff disease with platelet-rich plasma: a systematic review of randomized controlled trials. Arthrosc J Arthrosc Relat Surg. 2019;35(5):1584–1591. https://doi.org/10.1016/j.arthro.2018.10.115

King W, Toler K, Woodell-May J. Role of white blood cells in blood- and bone marrow-based autologous therapies. BioMed Res Int. 2018;2018:1–8. https://doi.org/10.1155/2018/6510842

Mirza MH, Bommala P, Richbourg HA, Rademacher N, Kearney MT, Lopez MJ. Gait changes vary among horses with naturally occurring osteoarthritis following intra-articular administration of autologous platelet-rich plasma. Front Vet Sci. 2016;3. https://doi.org/10.3389/fvets.2016.00029

Moraes APL, Moreira JJ, Brossi PM et al. Short- and long-term effects of platelet-rich plasma upon healthy equine joints: clinical and laboratory aspects. Can Vet J. 2015;56(8):831–838

Pochini AC, Antonioli E, Bucci DZ et al. Analysis of cytokine profile and growth factors in platelet-rich plasma obtained by open systems and commercial columns. Einstein (São Paulo). 2016;14(3):391–397. https://doi.org/10.1590/S1679-45082016AO3548

Rinnovati R, Romagnoli N, Gentilini F, Lambertini C, Spadari A. The influence of environmental variables on platelet concentration in horse platelet-rich plasma. Acta Vet Scand. 2015;58(1):45. https://doi.org/10.1186/s13028-016-0226-3

Schnabel LV, Mohammed 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. https://doi.org/10.2746/042516408X278030

Smit Y, Marais HJ, Thompson PN, Mahne AT, Goddard A. Clinical findings, synovial fluid cytology and growth factor concentrations after intra-articular use of a platelet-rich product in horses with osteoarthritis. J S Afr Vet Assoc. 2019;90. https://doi.org/10.4102/jsava.v90i0.1721

Smith JJ, Ross MW, Smith RKW. 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. https://doi.org/10.1055/s-0038-1632972

Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am J Sports Med. 2011;39(10):2135–2140. https://doi.org/10.1177/0363546511417792

Textor JA, Tablin F. Intra-articular use of a platelet-rich product in normal horses: clinical signs and cytologic responses: intra-articular use of a platelet-rich product in normal horses. Vet Surg. 2013;42(5):499–510. https://doi.org/10.1111/j.1532-950X.2013.12015.x

Tyrnenopoulou P, Diakakis N, Karayannopoulou M, Savvas I, Koliakos G. Evaluation of intra-articular injection of autologous platelet lysate (PL) in horses with osteoarthritis of the distal interphalangeal joint. Vet Q. 2016;36(2):56–62. https://doi.org/10.1080/01652176.2016.1141257

Wahl SM, Wong H, McCartney-Francis N. Role of growth factors in inflammation and repair. J Cell Biochem. 1989;40(2):193–199. https://doi.org/10.1002/jcb.240400208

Zhang J-M, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27–37. https://doi.org/10.1097/AIA.0b013e318034194e