Platelet-Rich Plasma in Regenerative Medicine

Joseph Alsousou and Paul Harrison

Abstract

The clinical application of platelet-rich plasma (PRP) has been increasing sharply in the last two decades. Its role as a potential regenerative agent and ease of application have allowed it to take huge share in the fast-evolving biological therapy field. The reported effect of PRP on a range of tissue types including bone, cartilage, tendon and muscle has attracted clinical interest in fields such as trauma, orthopaedic, maxillofacial and plastic surgery where effective healing of tissues is critical for successful outcome. The results of in vitro and animal studies that largely report positive effects of PRP on cellular and matrix regeneration have been the main drive for its translation to clinical settings. Despite the lack of appropriately powered trials, PRP administration remains an attractive strategy given its cost-effective, minimally invasive nature and the autologous nature of PRP. In this chapter, the current literature on the use of PRP in regenerative medicine is reviewed highlighting both some of the controversy surrounding this approach and some emerging clinical applications. A new PRP classification system is presented to allow better description of the variable clinical PRP products and their correlated outcome.

Introduction

Platelets are increasingly recognised to play important roles in physiology beyond promotion of haemostasis and thrombosis. Platelet-rich plasma (PRP), an autologous derivative of whole blood that contains a supraphysiological concentration of platelets, has gained increasing attention in both the scientific literature and the wider media for its potential application as a regenerative adjunct therapy (Alsousou et al. 2009; Engebretsen et al. 2010). The regenerative effect of PRP exerted by producing a local environment for tissue regeneration which is rich in growth factors and other cytokines has been supported by in vitro and animal studies that suggest a positive influence on the migration and proliferation of a number of cell types. Furthermore, the full array of potential bioactive growth factors and chemokines released upon platelet activation is becoming well defined (Coppinger et al. 2004; Nurden 2011). These include transforming growth factor (TGF-ß1 and TGF-ß2), platelet-derived growth factor (PDGF-AA, PDGF-AB and PDGF-BB), vascular endothelial growth factor (VEGFs A and C), insulin growth factor (IGF-1) and epidermal growth factor (EGF). These factors can promote local angiogenesis, stem cell homing and local cell migration, proliferation and differentiation, coupled with the deposition of matrix proteins such as collagen which all play a key role in enabling the restoration of normal tissue structure and function (Coppinger et al. 2004; Nurden 2011).

The reported clinical use of PRP is largely confined to the last two decades and initially centred on its application in dental and maxillofacial surgery (Gibble and Ness 1990; Sanchez et al. 2003). More recently, regenerative effects of

J. Alsousou, MD, D Phil
Institute of Translational Medicine, University of Liverpool, Liverpool L69 3BX, UK

P. Harrison, PhD (✉)
Institute of Inflammation and Ageing, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK
e-mail: p.harrison.1@bham.ac.uk

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PRP in a range of tissue types including bone, cartilage, tendon and muscle have attracted interest in fields such as orthopaedic and plastic surgery where effective restoration of sometimes poorly vascularised and damaged tissue is a critical determinant of successful clinical outcome (Alsousou et al. 2013; Gentile and Cervelli 2010). Despite the lack of appropriately powered trials, PRP administration remains an attractive strategy given its cost-effective, minimally invasive nature and the autologous nature of PRP. The latter eliminates concerns about the potential for disease transmission or immunological reactions. In this chapter, the current literature on the use of PRP in regenerative medicine is reviewed highlighting both some of the controversy surrounding this approach and some emerging clinical applications.

PRP Definition

Platelet-rich plasma (PRP), which is also known as platelet-enriched plasma (PeRP), platelet-rich concentrate (PRC), platelet concentrate, leucocyte-rich PRP (L-PRP), platelet-rich fibrin (PRF), platelet rich in growth factors (PRGF), platelet-rich fibrin matrix (PRFM), autologous concentrated plasma (ACP), platelet gel or platelet releasate, may be defined as a volume of the plasma fraction of autologous blood having a platelet concentration above baseline (Marx et al. 1998). A high concentration of (1,407,640 ± 320,100/μl) of platelets in plasma has been suggested to be a working definition of PRP (Weibrich et al. 2002). Although platelet-rich plasma refers to autologous preparations, the preparation of allogeneic PRP (aPRP) might not be excluded. The use of aPRP may serve as an alternative option in the case of patients who refuse to be subjected to a blood drawing procedure or have contraindications to autologous PRP (Dragica et al. 2007).

In clinical practice, there are a large number of PRP products obtained using different preparation methods. Although they are all labelled as PRP; the content, purity and hence the biological properties of those products all vary widely and could impact upon their potential efficacy. This is causing confusion among clinicians and scientists when it comes to reporting the clinical outcome of PRP applications. Several clinical trials have been conducted without clear quantification of the used PRP biological properties, which may differ significantly leading to varying outcomes. Table 1 shows a list of PRP preparation devices and the related product content. In this chapter, we will describe recently suggested classifications and then suggest a new system to classify PRP based on its biological components.

Cellular Components of PRP

PRP regenerative properties are based on the production and release of bioactive proteins including multiple growth and differentiation factors, following platelet activation. However, the role of other cells that may exist in PRP such as leucocytes and erythrocytes is still under investigation (Alsousou et al. 2010). The presence of leucocytes has a great impact on the biology of these products, not only because of their immune and antibacterial properties but also because they are key players in tissue healing and local factor regulation.

Most PRP products have varying concentrations of blood cells (platelets, leucocytes and erythrocytes) and cell-derived microvesicles, and many of them also have high concentrations of leucocytes. The direct cellular interaction between platelets, leucocytes and target tissues is still largely unknown (Bielecki et al. 2012). Polymorphonuclear leucocyte (PMN) and monocyte adhesion to activated platelets is important for the recruitment of PMN and monocytes at sites of tissue damage. The binding of activated platelets to PMN/monocytes involves P-selectin and the activated β_2-integrin CD11b/CD18 that not only results in transactivation but may cause significant changes in patterns of gene expression to trigger inflammation, e.g. tissue factor expression on monocytes. The effect of PRP components on cells and tissues is a complex process that involves growth factors and cytokines produced by both platelets and leucocytes. In addition, there may be direct cellular interactions that also regulate the intracellular signalling pathways in the resident tissue cells (e.g. ERK and AkT pathways for proliferation) (Franklin et al. 2012).

Bioactive Proteins and Growth Factors in PRP

Researchers have identified hundreds of potentially bioactive proteins either inside platelets or on the plasma membrane (Nurden 2011). The most commonly studied platelet proteins include PDGF, TGF-β, PDEGF, VEGF, IGF-1, FGF and EGF and some cytokines including proteins such as platelet factor 4 (PF4) and CD40L. Chemokines and newly synthesised metabolites are also released during platelet activation. PRP also facilitates the application of autologous plasma along with platelet-derived proteins. In addition, it promotes the development of a fibrin scaffold at the desired location that can act as a temporary matrix for cell growth and differentiation to assist repair in the injured tissue. Studying the role of those bioactive factors is essential to understand the biological activities of PRP.

Table 1 List of PRP preparation devices and their PRP products

Technology	Device name	Product name	Concentration increase[a]	Platelet recovery (%)	Product content
Floating buoy or shelf	Biomet GPS™	PCP	3.2×	70 %	Manual collection of buffy coat which contains platelets, WBC and minimal amount of RBC
	Harvest® SmartPRep2 BMAC™ Depuy Symphony II 3i PCCS	PRP	4.6× 4.0× 4.0×	65–72 %	
Cell saver-based systems	Electa, Haemonetics, CATS, BRAT	PRP	4–6×	75 %	Platelet concentrate only with plasma. No WBC or RBC contamination
Computer-aided system	Sorin Angel	PRP	4.3×	70 %	Automated buffy coat collection, which contains concentrated platelets, WBC fractions and minimal amount of RBC
	Arteriocyte Medical (Magellan™)	PRP	5.1×	76 %	
Standard centrifugation	AutoloGel System SmartPReP	PRP PReP	1–2×	78 %	Platelets in plasma suspension with minimum white cells and low concentration of platelets
	Cascade PRFM Fibrinet system	PRFM	1–2×	78 %	Platelet-rich fibrin membrane
	Choukroun's PRF	PRF	1–2×	70 %	Leucocyte- and platelet-rich fibrin
Direct siphoning	GenesisCS	PRP	6×	68 %	concentrate of platelets and leucocytes through siphoning device
Direct aspiration	Secquire Arthrex ACP	PRP	1.6×	31 %	Manual aspiration of platelet and plasma after centrifuging
Platelet separation	Vivostat	PRF FS	6×	65 %	Platelet-rich fibrin Fibrin sealant without platelet
Platelet filtration	Caption Advanced Tissue Regeneration (ART)-Curasan Set	PC	4.3×	–	Concentrated platelets without plasma

ACP autologous concentrated plasma, *PCP* platelet concentrated plasma, *PRF* platelet-rich fibrin, *PRFM* platelet-rich fibrin matrix, *PRGF* plasma rich in growth factors, *PRP* platelet-rich plasma, *RBC* red blood cells, *WBC* white blood cells

[a]Increase above baseline is based on a collection volume of 50–60 ml whole blood with a single pass (except for the cell saver device that uses 450 ml) (Alsousou 2014)

PRP Preparation

Preparation Methods

Autologous PRP can be prepared in a laboratory or an operating theatre or clinic room from anticoagulated blood collected in the immediate pre-therapeutic period. There are several choices of anticoagulants that can be used for blood collection. Trisodium citrate solution is the most widely used anticoagulant (citrate is a weak chelator of calcium that inhibits coagulation and platelet function) with few negative effects on PRP preparation (Alsousou et al. 2009). ACD (acid citrate dextrose) and CPD (citrate phosphate dextrose), with and without adenine (ACD-A and CPD-A), are also effective anticoagulants. The use of ethylenediaminetetraacetic acid (EDTA) is potentially more harmful in the preparation of PRP, and a large number of damaged/activated platelets have been observed during preparation with this material. Therefore it is not usually recommended for PRP preparation.

Traditionally, a relatively pure preparation and good yield of PRP can be easily obtained in a single step by low g centrifugation (170–200 g) of anticoagulated blood for 10 min at room temperature (Harrison et al. 2011a). A consensus of the working party from the Platelet Physiology Subcommittee of the Scientific and Standardisation Committee (SSC) at the International Society on Thrombosis and Haemostasis (ISTH) provides a g value recommendation for platelet preparation (Cattaneo et al. 2013).

For clinical applications there is no such standardisation, and there are three essential main methods available which can rapidly provide a sterile product suitable for clinical application: the (1) gravitational platelet sequestration (GPS) or centrifugation technique, (2) standard cell separators and (3) autologous selective filtration technology (plateletpheresis).

In the GPS method, three layers become evident: the bottom layer comprised of red blood cells (specific gravity = 1.09), the middle layer comprised of platelets and white blood cells (buffy coat, specific gravity = 1.06) and the top plasma layer (specific gravity = 1.03). Various methods to

collect the platelet layer have been developed, but with varying degrees of red and leucocyte contamination (Table 1). Platelet concentration, yield and recovery are also dependent on the centrifugation protocol and collection method (Kevy and Jacobson 2004). One study evaluated the effect of different centrifugal forces and showed that centrifugation at greater than 800 g may actually decrease the amount of releasable TGF-β from the PRP (Landesberg and Glickman 2000).

Standard cell separators and salvage devices generally operate on a full unit of blood. In general, they use continuous flow centrifuge bowls or continuous flow disc separation technology coupled with hard and soft centrifugation steps. Weibrich et al. described a discontinuous technique with a cell separator that produces a fivefold increase in platelet count (Weibrich et al. 2002). The red blood cells and some, or all, of the platelet-poor plasma (PPP) can also be returned to the patient to maintain circulating volume.

Selective filtration technology or plateletpheresis depends on a single-use proprietary filter designed to concentrate platelets from whole blood. The platelets are captured on the filter and are then harvested to provide a platelet-rich concentrate (PRC). Although this technique reduces platelet lysis, which may be induced during centrifugation, platelet fragmentation and activation have also been shown to occur within filtration-based systems (Schoendorfer et al. 1990; Kevy and Jacobson 2004).

In general, most systems do not concentrate the plasma proteins of the coagulation cascade (Kevy and Jacobson 2004). Plasma protein concentrations above baseline can be achieved through secondary ultrafiltration. The autologous growth factor filter (AGF) is a microporous, hollow, cellulose fibre filtration device with a volume of 8 ml and uses PRP as a baseline product. The device filters water after multiple passes of PRP through the filtration device. As much as two-thirds of the aqueous phase is removed by filtration, thus increasing the concentrations of the retained plasma proteins and formed elements (Everts et al. 2006). Evidently each preparation technique will also result in significant differences in yields, concentration, purity, viability and activation status of the isolated platelets. Each of these variables will therefore not only influence the eventual concentrations of the bioactive proteins released from the platelet granules but also affect the eventual clinical efficacy of each PRP preparation (Whitman et al. 1997).

Handling and Activation of PRP

Once the PRP is prepared, it is claimed that it is stable for clinical use, in the anticoagulated state, for 8 h or longer (Marx et al. 1998). Some authors advocate using PRP within no longer than 6 h if it is kept at room temperature (Ho and Chan 1995). However, Sweeney et al. (2006) suggested using a specific method, which included adding low-pH glucose-containing additive solution with $NaHCO_3$ to platelets within ELX bags, to try and keep PRP viable for up to 7 days by lowering the pH to allow sterilisation and provide energy to maintain platelet viability.

PRP must also be activated for the platelets to release their α-granule contents, with the clot providing a vehicle to capture the secreted proteins and maintain their presence at the application site. Activation with bovine thrombin is no longer recommended as this was reported to cause coagulopathy resulting from cross reactivity of anti-bovine factor V antibodies with human factor V (Cmolik et al. 1993; Landesberg et al. 1998). Calcium chloride is therefore a much safer, but slower, alternative and without any risk of prion or pathogen exposure. Platelet activation via this method takes at least 20 min (Anitua et al. 2004). Recently, autologous thrombin preparation kits have been introduced as a safe and faster activation alternative (Man et al. 2001). Furthermore, injecting inactive PRP into collagen rich tissues may also result in direct platelet activation resulting in degranulation (Fufa et al. 2008; Harrison et al. 2011b). Harrison et al. found that thrombin-activated platelets lead to immediate release of TGF-β1 and PDGF-AB, while the collagen-activated PRP clots resulted in 80 % greater cumulative release of TGF-β1, VEGF and PDGF than thrombin over 7 days (Harrison et al. 2011b).

In Vitro and Animal Studies of PRP

Cell and Tissue Culture Studies

PRP influences the migration, proliferation and differentiation of a number of cell types, although the temporal features and molecular basis of this effect remain unclear. Castelnovo et al. (2000) demonstrated an 80-fold enhancement of porcine retinal cell migration 48 h after the application of human platelet suspension, and PRP has been shown to promote the recruitment of circulation-derived cells for tendon healing in vivo (Kajikawa et al. 2008). PRP can also have a mitogenic effect on endothelial cells (Bertrand-Duchesne et al. 2010), bone (Bertoldi et al. 2009), cartilage (Akeda et al. 2006; Ishida et al. 2007), periodontal ligaments (Pantou et al. 2010) and mesenchymal and dental stem cells (Feng et al. 2010; Huang and Wang 2010). However, there are conflicting reports concerning the effects of PRP on cell differentiation. While several studies indicate a positive influence (Bertoldi et al. 2009; Huang and Wang 2010; Lee et al. 2011; Lucarelli et al. 2005), PRP has been shown to inhibit the osteogenic differentiation of bone marrow-derived pre-osteoblasts in a dose-dependent manner (Arpornmaeklong et al. 2004) and has an inhibitory effect

on mesenchymal stem cell differentiation when applied in isolation, but a stimulatory effect with 1,25(OH)2vitamin D3 co-treatment (Feng et al. 2010).

The effect of PRP on cellular activity appears to be highly time and dose dependent. Soffer et al. (2004) showed that while short-term exposure to human platelet lysate (<24 h) promotes bone cell proliferation and chemotaxis, long-term exposure results in decreased levels of alkaline phosphatase and mineral formation. Other studies indicate that PRP stimulates the growth of bone marrow cells for 4–8 days (Van Den Dolder et al. 2006) and that, beyond 6 days, washed platelet solution has a stronger influence on mesenchymal cell proliferation than PRP (Duan et al. 2011). The concentration of PRP also appears to be critical in determining its effect. Some authors suggest that high concentrations adversely affect fibroblast cell viability and proliferation (Choi et al. 2005; Creeper et al. 2009). Cheng et al. demonstrated the significance of both the platelet and plasma protein constituents of PRP as these fail to significantly enhance gene expression of collagen types 1 and 3 in cultured ligament cells when applied in isolation, but do succeed when in combination (Cheng et al. 2010).

Several studies have confirmed the ability of PRP to induce local gene expression. The growth factors TGF-β1 and PDGF appear to play an important role in tendon and ligament regeneration by promoting expression of proteins including collagen and cartilage oligomeric matrix protein (Cheng et al. 2010; Lyras et al. 2010c; Schnabel et al. 2007). Histologically, PRP has been shown to promote gross ligamentisation of tendons (Sanchez et al. 2010) and increase the maximum breaking strength and stiffness of healing tendons in vitro when used with a bone marrow-derived stromal cell-seeded graft (Morizaki et al. 2010). Activation of the Nrf2-antioxidant response element pathway has been implicated in the increase in tenocyte growth due to addition of platelet-released growth factor (Tohidnezhad et al. 2011). Further, Lyras et al. (2010b, 2011) showed that a single application of PRP to the Achilles and patellar tendon defects significantly enhances IGF-1 protein expression and is associated with a faster healing process confirmed by histology (thicker tendons with organised fibre structure). Anitua et al. demonstrated that PRP application results in increased expression and production of VEGF and HGF in cultured tenocytes (Anitua et al. 2005). Other in vitro studies confirmed that the ability of PRP to promote angiogenesis, which is crucial in acute tissue injury repair, may be an important reason for its regenerative effects (De Mos et al. 2008; Lyras et al. 2010a; Lyras et al. 2009).

It is unclear whether the mitogenic effects of PRP are dependent upon the presence of intact platelets or not. Lysed solutions have been shown to have a positive effect on osteogenic activity (Slater et al. 1995) and bone marrow stem cells (Oprea et al. 2003), but other studies also suggest that unviable or damaged platelets after processing may have impaired growth factor secretion (Marx 2001, 2004).

In Vivo Animal Studies

PRP has been shown to promote bone regeneration in rat and rabbit calvarial defects (Mariano et al. 2010), the alveolar sockets of *Cebus apella* monkeys (Pessoa et al. 2009) and porcine tibial metaphyseal defects (Jungbluth et al. 2010), as well as promote the incorporation of mandibular bone grafts in goats (Fennis et al. 2002). However, other studies suggest that PRP has no effect on bone healing and may actually be detrimental when used as a supplement to bone grafts. In a study looking at craniofacial bone repair in rabbits, PRP-treated grafts were associated with diffuse fibrous tissue deposition that inhibited bone formation when compared with untreated grafts (Giovanini et al. 2011). Broggini et al. also showed that PRP alone, or when used in combination with autogenous bone, failed to promote remodelling in rabbit calvaria, whereas autogenous bone without PRP leads to accelerated remodelling (Broggini et al. 2011).

Transected rat Achilles tendons treated with PRP showed increased callous strength and stiffness by 30 % after 1 week (Aspenberg and Virchenko 2004). Similar effects were observed on lesioned equine superficial digital flexor tendons treated with PRP with significantly higher strength at failure, improved collagen network organisation and increased metabolic activity than saline-treated controls (Bosch et al. 2010). Other animal and in vitro studies have also suggested a key role for angiogenesis as a mechanism underlying the regenerative effects of PRP (Anitua et al. 2005; Bir et al. 2011; De Mos et al. 2008; Li et al. 2009; Lyras et al. 2010a). In a porcine model, Hadad et al. showed that adipose-derived stem cells and PRP significantly enhance healing of perfusion-depleted tissue when applied in combination, but not in isolation, lending support to the hypothesis that the growth factors contained in activated PRP have a permissive effect in enabling regeneration (Hadad et al. 2010).

Animal studies have also shown that PRP application improves left ventricular function following ischaemia reperfusion (Mishra et al. 2011), promotes fat graft survival (Nakamura et al. 2010) and enhances the repair of the cartilage (Milano et al. 2010), skin (Derossi et al. 2009), corneal epithelium (Tanidir et al. 2010), liver (Matsuo et al. 2011), intestine (Yamaguchi et al. 2012) and peripheral nerves (Yu et al. 2011).

Clinical Studies of PRP

Many clinical research studies are either predominantly case studies or limited case series. There have been several randomised trials (RCT) investigating the use of PRP, with a recent systematic review and meta-analysis identifying 23 trials in 13 conditions (Sheth et al. 2012). Pathologies studied include arthroplasty, spinal fusion and a range of tendon pathologies. The 2012 review used the GRADE evaluation method and concluded that the quality of evidence is very low. The authors concluded 'the current literature is complicated by a lack of standardization of study protocols, platelet separation techniques, and outcome measures'. However, despite this uncertainty about the evidence, there is increasing clinical use of PRP within clinical practice in many areas with sports medicine being the most popular.

Bone

There are few clinical studies examining the role of PRP in bone healing after orthopaedic trauma (Alsousou et al. 2009; Pietrzak and Eppley 2005). Most of the published studies are related to the use of PRP in oral and cranial surgery. Currently, it is not uncommon to combine the platelet-rich material with autograft, allograft, demineralised bone matrix or other graft material to fill bony defects in the mandible or cranium (Marx et al. 1998).

In small cohort studies, percutaneous injection of autologous platelet–leucocyte-rich gel has been shown to be an effective and less invasive alternative to bone marrow injection in the treatment of delayed bone union (Bielecki et al. 2012) to promote faster fusion in spinal surgery (Hartmann et al. 2010; Lowery et al. 1999) and healing of clavicle fracture nonunion (a single case) (Seijas et al. 2010).

In bony defects, PRP reduces the time taken for bone regeneration (Nagaveni et al. 2010; Rodrigues et al. 2011) and has been used to treat mandibular osteonecrosis resulting from radiotherapy (Scala et al. 2010) or the use of bisphosphonates (Curi et al. 2007). Cenni et al. recently provided a possible biological explanation for this through the demonstration that growth factor release from platelets is maintained within regions of osteonecrosis (Cenni et al. 2011). In one series, researchers were able to measure levels of PDGF and TGF-β in the fracture haematoma of 24 patients who had fresh fractures of the foot and ankle. However, the investigators were unable to detect these same proteins in the nonunion tissue of seven patients presenting with similar fractures. After PRP application of the nonunion during revision surgery, radiographic union was observed by an average of 8.5 weeks (Gandhi et al. 2005).

The use of PRP in maxillofacial surgery is well supported with recently published level 1 and 2 evidence (meta-analysis or randomised controlled trials). However, there is no level 1 or level 2 evidence showing any cortical bone benefits from PRP. There are just a few anecdotal reports on the effect on a mix of corticocancellous bone or a mixture of PRP and bone substitutes.

Wound Healing

As early as 1990, autologous human platelet-derived wound healing factors (HPDWHF) were proposed to regulate wound healing of recalcitrant skin ulcers by promoting the formation of granulation tissue in the early healing phase (Herouy et al. 2000). PRP and other platelet-rich products have been shown to promote healing in a range of cutaneous lesions (Crovetti et al. 2004; Lacci and Dardik 2010; O'connell et al. 2008) and have therefore attracted interest in fields such as plastic surgery where structured approaches to the use of PRP are beginning to emerge (Gentile and Cervelli 2010). In a randomised controlled trial, diabetic foot ulcers treated with PRP gel showed significantly reduced time to healing compared with controls (Driver et al. 2006). While there have been reports of negative results with the use of platelet-derived wound healing factors in ulceration, most notably in a trial by Krupski et al. (1991), more recent studies using autologous PRP have supported its beneficial role in chronic wound healing and provided evidence that it may actually reverse non-healing trends (Frykberg et al. 2010; Lacci and Dardik 2010).

Post-traumatically, PRP promotes ulcer healing (Cervelli et al. 2011a) and faster re-epithelialisation of lower extremity wounds with bone exposure (Cervelli et al. 2011b) and, in combination with hyaluronic acid, can act as an effective scaffold for cellular growth in covering exposed tendons in acute and chronic open wounds of the foot and ankle (Cervelli et al. 2010). In a 5-year study assessing the effect of applying platelet gel to patients presenting with traumatic loss of finger substance, Balbo et al. reported better aesthetic results and a shorter recovery time in the PRP-treated group (Balbo et al. 2010). However, although an early case report suggested that PRP promotes healing even in the presence of infection (Cieslik-Bielecka et al. 2009), recent randomised studies have shown that it fails to actually prevent infection if administered during inguinal surgery (Lawlor et al. 2011) or during wound closure following venous harvesting (Almdahl et al. 2011).

Marck et al. recently performed a systematic review of the use of PRP in burn injury (Marck et al. 2014). They concluded that the literature on the use of PRP in burns is scarce. Despite this growth factors have been shown to be beneficial, and both animal studies and case reports have

shown that PRP can improve healing times post burn injury. Deep dermal burns may also benefit from the pro-haemostatic effects, antimicrobial properties and well-known positive effects of platelets on wound healing. However, patients with large burns also have profound changes to their physiological state including changes in platelet counts observed post injury, and it is unknown how this may affect the quality of autologous platelet preparations (Marck et al. 2013). Also it is largely unknown how PRP may affect long-term scarring given that platelet growth factors such as TGF-β are implicated. Overall they concluded that further research is required to study the potential efficacy of PRP in burns. One of the obvious problems in burn injury is how to apply activated PRP gel evenly over a large surface area of injury while maintaining biological activity.

Use of PRP in Tendon Disorders

In pilot studies, PRP has been shown to reduce pain in patients with chronic tendinopathy (Gaweda et al. 2010; Kon et al. 2009), with a persisting effect for at least 2 years from the time of treatment despite normalisation of VEGF, EGF and CCL2 blood levels after 24 h (Volpi et al. 2010). More recently, in the 2-year follow-up of a double-blind randomised controlled trial comparing PRP with corticosteroid injection for the treatment of chronic lateral epicondylitis, Gosens et al. reported significantly increased function and reduced pain in the PRP-treated group compared to those treated with steroids (Gosens et al. 2011). The benefit of PRP as a second-line, surgery-sparing therapy in the treatment of elbow tendinopathy resistant to physical therapy has been further demonstrated in a prospective randomised trial reported earlier this year (Creaney et al. 2011). Creaney et al. study showed that injecting PRP in chronic tendinopathy reduced the need for surgery from 20 to 10 % when compared with autologous blood injection alone.

Despite the evidence that PRP may be effective in treating tendinopathy, there is also a growing body of evidence to the contrary. The most significant trial in this regard was a double-blind randomised controlled trial carried out by De Vos et al. which showed no difference in pain or function in patients with chronic Achilles tendinopathy treated with PRP compared with those treated with saline (De Vos et al. 2010b). A recent study identified no significant difference in the degree of neovascularisation or tendon structure (using colour Doppler ultrasonography and ultrasonic tissue characterisation) in patients with chronic Achilles tendinopathy treated with PRP compared with those receiving a saline placebo (De Vos et al. 2011). Another application of PRP that has gained significant attention is its use in anterior cruciate ligament (ACL) reconstruction using hamstring tendon grafting following traumatic injury. In a randomised, double-blind trial, Vogrin et al. used contrast-enhanced MRI to demonstrate that ACL grafts treated with platelet gel had a significantly higher level of vascularisation at the osteoligamentous interface 4–6 weeks postoperatively compared to control grafts (Vogrin et al. 2010b). PRP gel has also been shown to reduce the time taken for a homogenous appearance on MRI following ACL reconstruction by 48 % (Radice et al. 2010), although contrary to this Silva and Sampaio demonstrated a failure of PRP to affect the signal intensity of the fibrous interzone in femoral tunnels following grafting (Silva and Sampaio 2009). Functionally, the application of platelet gel to grafts has been shown to significantly improve anteroposterior knee stability following surgery (Vogrin et al. 2010a).

With regard to the use of PRP in treating rotator cuff tears, a pilot study of patients undergoing arthroscopic rotator cuff repair showed that intraoperative PRP administration was associated with significantly increased function and reduced pain at 6, 12 and 24 months postoperatively with no adverse events (Randelli et al. 2008). However, a recent randomised controlled trial demonstrated that platelet-rich fibrin matrix has no benefit in enhancing healing of small- and medium-sized rotator cuff tears (Castricini et al. 2011): as with tendinopathy, the consensus in the literature is that further trials are needed to determine whether neutral results regarding the benefit of platelet-rich mediums indicate a lack of benefit from having additional growth factors in certain healing environments or whether they reflect suboptimal preparation and administration techniques (Engebretsen et al. 2010; Hamilton et al. 2010). Two recent reviews conclude that the poor methodological quality of trials assessing the use of PRP in treating chronic tendinopathy means the evidence supporting its effectiveness is limited and thus insufficient to recommend its routine clinical use (De Vos et al. 2010a; Kampa and Connell 2010).

A thorough literature search of the application of PRP in Achilles tendon rupture in both animal and human studies was recently conducted. Nine studies present results for the use of platelets in Achilles tendon rupture treatment, seven from animal experiments and two from human trials (Sanchez et al. 2007; Schepull et al. 2011). Six of the animal studies used a rat model, and one used an ovine model. All animal studies, using biomechanical and histological assessments, are in agreement showing a beneficial effect of platelets. Only two human studies have tested the effect of platelets on the treatment of Achilles tendon rupture based on imaging techniques and clinical results. Sanchez et al. (2007) found positive effects in a group of 12 athletes treated with PRP-augmented suture repair at 32 months in a case–control study. In ultrasonographic assessment, there was less tendon thickening and higher concentrations of TFG-beta and other growth factors, and patients regained range of

motion faster and returned to sports (gentle running) earlier. In a randomised study of 30 patients, Schepull et al. (2011) found no effect of platelets on radioisometrical tendon contraction or clinical outcomes. Both of these underpowered clinical studies used PRP as an adjunct to open surgical repair, and this may have obscured any effect of PRP on healing.

There are three other studies that report on the use of platelets in human Achilles tendinopathy although the results of these trials have little bearing on Achilles tendon rupture, which has a very different pathology, regenerative properties and treatment pathways. Therefore there is only one underpowered randomised clinical trial that has assessed PRP in Achilles tendon rupture (Sanchez et al. 2007; Schepull et al. 2011), and the authors of that trial recognised a limitation in their platelet preparation technique and storage (of up to 20 h) resulted in only a 20 % release of growth factors from the platelets. In our own 20-patient pilot study, we achieved an average of 69 % growth factor content release after stimulation.

Recently published systematic reviews (Sadoghi et al. 2012; Taylor et al. 2011) have concluded that there are encouraging signs that PRP could be developed as an effective therapy. Sadoghi et al. (2012) concluded that there is evidence in support of a positive effect of platelet concentrates in the treatment of Achilles tendon ruptures in vivo in animal models and human application, consistent with a medium- to large-sized effect. This effect is most likely attributed to enhanced scar tissue maturation. In another systematic review, Taylor et al. (2011) concluded that PRP use in tendon and ligament injuries has several potential advantages, including faster recovery and, possibly, a reduction in recurrence, with no adverse reactions described. One ongoing randomised trial is studying the effect of PRP on ruptured Achilles tendon (Anonymous 2015); the PATH-2 trial will study the efficacy and mechanism of PRP in pragmatic settings which include clinical, histological and laboratory outcome measures, as well as measurement of platelet quality, purity and growth factor content (Anonymous 2015).

Potential Adverse Risks of PRP

PRP is prepared from autologous blood; therefore it is inherently safe, and any concerns of disease transmission such as HIV, hepatitis or Creutzfeldt–Jakob disease or immunogenic reactions that exist with allograft or xenograft preparations are eliminated (Alsousou et al. 2009). However, using bovine thrombin preparations, which contain bovine factor V to activate the platelets, may lead to immunogenic reactions. The systemic use of bovine thrombin in cardiovascular surgery to promote clotting has been reported to be associated with coagulopathies resulting from cross reactivity of anti-bovine factor V antibodies with human factor V (Cmolik et al. 1993; Landesberg et al. 1998). The bovine thrombin preparations used in those reported cases were high dose (>10,000 units) and were applied directly onto open wounds where absorption into systemic circulation is certain. There have been no similar reports since 1997 due to the use of highly purified bovine thrombin. Conversely, the very small dose of bovine thrombin (<200 units) used to activate PRP before application will be consumed during clot formation and digested by macrophages. Hence, bovine thrombin-activated PRP is unlikely to produce anti-factor V antibodies.

The use of bovine thrombin for activation is therefore now avoided in modern preparation techniques. Some authors proposed that PRP gel formation can be performed only with the addition of calcium chloride, but this usually takes longer to complete (Marlovits et al. 2004). An effective alternative is thrombin receptor agonist peptide (TRAP) that mimics the effect of thrombin on PAR-1 receptors. Most current techniques prefer to use autologous thrombin or calcium chloride to activate PRP.

To date, there is no compelling evidence of any systemic complications of local PRP injection. Furthermore, there are no scientific reports suggesting potential cause–effect relationships between growth factors present in PRP and an increased risk of carcinogenesis. This may be because of the limited need for PRP injections (as PRP is not chronically administered) and because of the short in vivo half-lives and local bioavailability of growth factors produced by PRP.

Clinical Trials

Based on the inconclusive results in the current literature, the authors cannot provide solid evidence in favour of the clinical application of PRP. However, because the majority of the reviewed clinical trials reported encouraging outcomes, further controlled clinical trials are warranted to elucidate where PRP should be used.

However, clinical trials should include a sufficient number of patients and a proper design (randomised controlled trials), including a test group similar to control, except for the application of PRP. Also, proper description of the PRP procurement techniques must be included, including the amount of blood collected, baseline number of platelets, yield, purity, quality and growth factor content of PRP obtained, contaminating cells, concentration factor of platelets above baseline, coagulation promoters, doses and mixing ratio to PRP.

Clinical Study Design

Given the rudimentary knowledge of the mechanism of action of PRP in clinical settings and the limitations of current studies, it is important that any future clinical trial should be carefully designed not only with adequate power to accurately determine the effect of PRP on any particular tendon disorder but also to use disease-specific outcome tools. This has been highlighted by several authors (De Vos et al. 2010a; Maffulli and Del Buono 2012), who have shown that the imprecision in effect size estimates from underpowered studies investigating the use of PRP has led to unsafe conclusions. A study powered to determine if there are clinically important effects while furthering the understanding of PRP mechanism in clinical settings would add significantly to our understanding of the appropriate clinically used PRP. In a consensus paper published recently (Engebretsen et al. 2010), a panel of experts listed important elements of clinical trials of PRP: (1) RCT design, (2) clear inclusion/exclusion criteria, (3) homogenous study population or stratification of variables, (4) standardised clinical assessment, (5) validated PRP production method and delivery, (6) robust outcome measures and (7) standardised post-treatment follow-up protocol.

Currently, we are only aware of one ongoing randomised clinical trial that includes all the above and is adequately powered to measure the efficacy of PRP in clinical settings; the PATH-2 trial has started recruiting patients with acute Achilles tendon rupture and is due to finish in 2018 (Anonymous 2015).

PRP Classification System

'Not all PRP is in fact PRP'

In the field of platelet concentrates, most products are termed PRP. Unfortunately, this term is very general and incomplete, leading to confusion in the scientific literature and misleading conclusions. Currently, there are more than 12 platelet concentrate products available commercially, and although they have varying preparation methods and components (Table 1), they are all labelled PRP.

Accurate use of the terminology to reflect the product properties is required in both clinical and scientific applications. The terminology must be simple, accurate and pragmatic. It also must avoid commercial interests and thus remain scientifically homogeneous (Dohan Ehrenfest et al. 2012). Clinically prepared PRP is in fact a term that includes a wide range of products that have varying biological components (Dohan Ehrenfest et al. 2009). Most of these products have varying concentrations of blood cells (platelets, leucocytes and erythrocytes) and microvesicles. Two authors have tried to classify PRP products according to their biological content, which are described below.

Dohan Ehrenfest et al. (2009) classified the different platelet concentrates into four categories, depending on their leucocyte and fibrin content: pure platelet-rich plasma (P-PRP), leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF) and leucocyte- and platelet-rich fibrin (L-PRF) (Table 2). Yet, Mishra et al. (Mishra et al. 2012) used the presence of leucocytes and the method of activation to classify PRP into four types (Table 3).

In addition to the aforementioned factors, platelets are very sensitive to in vitro manipulation and can be easily activated during preparation methods leading to the premature release of their bioactive contents before application to the targeted tissues (Alsousou et al. 2013, 2009; Harrison 2004). Therefore details of the preparation method and ultimate platelet quality (e.g. concentration, viability, activation status and growth factor content) may also be important to potentially stratify any clinical effects.

The authors of this chapter suggest a new classification system, which incorporates the above two classification systems and includes the preparation method technique. This new system will use a combination of numeric and alphabetical characters to identify the class of PRP product. In addition to the two identifiers in the Ehrenfest and Mishra classifications, leucocytes and fibrin, our proposed new system will also include further identifiers activation, platelet concentration and preparation method categories.

Table 2 Ehrenfest classification system of the main available PRP products (Dohan Ehrenfest et al. 2009)

PRP class	Leucocytes	Fibrin content
P-PRP	No	Low
L-PRP	Yes	Low
P-PRF	No	High
L-PRF	Yes	High

Table 3 Mishra classification is divided into four types (1–4) and two subtypes (A and B) (Mishra et al. 2012)

	White cells	Activation	Platelet concentration
Type 1	Increased	No activation	A >5× B <5×
Type 2	Increased	Activated	A >5× B <5×
Type 3	Minimal or no WBC	No activation	A >5× B <5×
Type 4	Minimal or no WBC	Activation	A >5× B <5×

Table 4 The modified PRP classification system. The subcategories of activation, platelet concentration and preparation technique are added after the main class (e.g. L-PRP IB1) (Alsousou 2014)

Class	Leucocytes	Fibrin	Activation	Platelet concentration	Preparation category
P-PRP	–	Low	I II	A B C	1 2 3
L-PRP	+	Low	I II	A B C	1 2 3
P-PRF	–	High	I II	A B C	1 2 3
L-PRF	+	High	I II	A B C	1 2 3

Activation is divided into two subcategories:

I for the use of activation
II for PRP application without activator

Unlike the Mishra classification, platelet concentrates are subdivided into three categories (A, B and C) based on the range of platelet counts in the samples. This is in line with evidence presented previously in this chapter (Weibrich et al. 2004), which states that the most effective platelet count range is 900–1700 $\times$ 10^3/μl. The three categories are:

A. Platelet count $<900 \times 10^3$ /μl
B. Platelet count 900–1700 $\times$ 10^3/μl
C. Platelet count $>1700 \times 10^3$/μl

Furthermore, the preparation methods are classified into three categories:

1. The gravitational platelet sequestration (GPS) technique
2. Standard cell separators
3. Autologous selective filtration technology (plateletpheresis)

This modified classification is presented in Table 4. For example, the PRP used in conducting our PATH-2 trials will be classified as L-PRP IB1, which indicates that this PRP is prepared using the GPS method, which contained leucocytes with a platelet count range of 900–1700, and denotes that the platelets have been activated before application.

Conclusion

The role of PRP in tissue regeneration and its clinical application are a fast-developing field. Although, the effect of PRP on cell lines and tissues has been studied both in vitro and in animal models, clinical applications are still lacking the level of evidence from purposely designed randomised controlled trials. Further controlled clinical trials to elucidate the effects of PRP in pragmatic settings are warranted that need to take into account the quality control of the various PRP preparations utilised to ensure that optimal relationship between the product and outcome is established. Further studies into the mechanism of PRP tissue regeneration may also help elucidate the optimum combination of bioactive factors required to achieve best clinical efficacy.

Take-Home Messages

- The regenerative effect of PRP exerted by producing a local environment for tissue regeneration which is rich in growth factors and other cytokines has been supported by in vitro and animal studies.
- The clinical application of autologous PRP in regenerative medicine has become very popular in many areas of medicine. Despite this, there is a lack of adequately powered trials to provide solid evidence for its clinical use.
- Clinical preparations of PRP are heterogeneous and are often poorly standardised and defined.
- Future trials should be adequately powered to include a sufficient number of patients and controls and include a full description of the PRP preparation methodology utilised as well as the yield, purity, quality and growth factor content of the PRP used.
- We present a new classification system of PRP taking into account the actual content (e.g. with leucocytes and fibrin), the activation methodology, the platelet concentration obtained and the preparation method used.
- Further clinical trials in this area are warranted taking into account the quality of the various PRP preparations utilised to enable a link between sample quality and clinical outcomes, which is also to be established.

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