PLATELET RICH PLASMA IN TENDINOPATHIES

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Tendinopathy is a broad term encompassing painful conditions occurring in and around tendons in response to overuse. There is currently no consensus on its optimal treatment. Conservative management is the first line treatment in most patients by activity restriction, non-steroid anti-inflammatory drugs, physiotherapy and judicious use of orthoses. However, these traditional treatments fail in some patients. These subsets of patients are treated with steroid injections or surgical interventions but there is no assurance of relief. In such cases a novel emerging technique involving injection of platelet rich plasma isolated from the patient's own blood, at the site of injury, is proving to be more effective. Although there is still insufficient evidence to support the use of PRP for treating musculoskeletal injuries, due to the ease of its preparation and minimal side effects on application, PRP has received much attention as a promising treatment option and the clinical use of PRP is increasing. Platelet-rich plasma (PRP), also known as platelet-enriched plasma (PeRP), platelet-rich concentrate (PRC), autogenous platelet gel, or platelet releasate, may be defined as the volume of the plasma fraction of autologous blood having a platelet concentration above baseline.[1] Normal platelet counts in blood range between 150,000/µl and 350,000/µl and average about 200,000/µl.[2]

Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cell, which are formed in the bone marrow. They are the smallest of the blood cells, round or oval in shape and approximately 2µm in diameter. They lack nuclei but contain organelles such as mitochondria, microtubules, and secretory granules (α, δ, λ). Among the three types of platelet secretory granules, α-granule is the most abundant and essential to normal platelet activity. These granules are formed during maturation of the megakaryocytes. They are about 200 nm to 500 nm in diameter, and approximately 50 to 80 granules are formed in each platelet.[3] α-granules contain more than 30 bioactive proteins, many of which have a fundamental role in hemostasis or tissue healing. Hemostasis can be considered to be the first stage of healing of injured tissue.[4]

The properties of PRP are based on the production and release of multiple growth and differentiation factors when the platelets are activated. Platelets begin actively secreting these proteins within ten minutes of clotting,[5] with more than 95% of the pre-synthesized growth factors secreted within one hour.[6] After the initial burst of growth factors, the platelets synthesize and secrete additional such factors for the remaining several days of their life span. These growth factors secreted mainly by alpha granules are known to cause tissue healing by promoting cellular chemotaxis, proliferation and differentiation of cells, removal of cellular debris , vascular invasion by angiogenesis and formation of extracellular matrix.[7] Since PRP is derived autologously from the patient's own blood there are no concerns about immunological reactions and disease

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transmission associated with other allergenic biological treatments like fibrin glue. Recombinant tissue growth factors developed by bioengineering have been used previously to obtain similar results but they have short shelf lives and are expensive which has precluded their wide spread use in routine clinical practise. Also, unlike recombinant growth factors, PRP delivery has the advantage of releasing multiple growth factors and differentiation factors upon platelet activation.[8]

The objective of this article is to review the current literature on the role of PRP in the treatment of tendinopathies and identify possible difficulties and complications associated with its use.

METHODS

A MEDLINE search was done with the key words Platelet Rich plasma and tendon and original research articles with free full-text were analyzed.

PREPARATION OF PRP

PRP can be prepared within minutes in a laboratory, an operating theatre or a clinic from blood collected in the immediate period before treatment. Usually, citrate or Anticoagulant Citrate Dextrose Solution, Solution A, USP (2.13% free citrate ion) (ACD-A) anticoagulation is highly recommended because it better preserves blood and ex vivo platelet reactivity. In contrast, EDTA and heparin should be avoided due to decreased platelet reactivity leading to reduced release of growth factors.[9,10]

For collection of PRP, automated blood cell separators use microprocessors to draw and anticoagulate blood, separate components either by centrifugation or by filtration, collect the desired component and recombine the remaining components for return to the donor. Centrifugal separation involves either a discontinuous or a continuous flow of blood into the devices. There are at least three techniques for preparation : the gravitational platelet sequestration (GPS) technique, standard cell separators, and autologous selective filtration technology (plateletpheresis).[11]

The GPS is a table-top centrifuge system using a flat-bottomed, 60-ml plastic centrifuge tube containing a buoy. PRP is collected following a 12-minute spin at 3200 rpm. When anticoagulated blood is centrifuged, three layers become evident. The bottom layer is comprised of red blood cells (specific gravity = 1.09), the middle of platelets and white blood cells (buffy coat, specific gravity = 1.06), and the top of plasma (specific gravity = 1.03). The buoy is lowered to remove the platelet-poor plasma, thereafter, PRP (about 5ml) volume is collected. PRP yield is approximately 10% of the volume of whole blood drawn. With this device, the red blood cells cannot be collected separately and are therefore discarded.

Standard cell separators and salvage devices generally operate on a full unit of blood. In general, they use a continuous-flow centrifuge bowl or a continuous-flow disk separation technique. It uses both hard spin (5,400-rpm) and soft spin (2,400-rpm) to collect the PRP. PRP is collected using a syringe. Weibrich and Kleis[18] describe a discontinuous technique with a cell separator that also produces a fivefold increase in platelet count. In both cases, the red blood cells and some, or all, of the platelet-poor plasma (PPP) can be retransfused to the patient to maintain circulating volume. Small compact office systems have been developed that produce approximately 6 ml of PRP from 45 ml to 60 ml of blood, obviating the need for reinfusion.[18,20] These systems differ widely in their ability to collect and concentrate platelets, collecting from 30% to 85% of the available platelets and increasing the platelet concentration between two and eight fold.[19] Some of the units permit the processing of two sets of disposables at once, or performing multiple sequential processes using the same disposable set, so that multiples of the 6 ml standard volume of PRP can be produced.

While preparing PRP by either of the above techniques, it is important that centrifugation be sterile and precisely suited to separating platelets from red blood cells with adequate concentrations of platelets.[12] Not all currently available

commercial devices are same, and some probably do not concentrate active platelets in sufficient numbers to enhance healing. This might explain the variability of the clinical efficacy of PRP. Studies suggesting that there is no benefit from PRP might be based on a product of poor quality produced by inadequate devices. Several studies suggest different centrifugation cycles in terms of time and force (4). The centrifugation force may be a critical step in preparation of PRP as applied mechanical forces may damage platelets, thereby losing the granular load of the growth factors. One study evaluated the effect of different centrifugal forces and showed that spins > 800 g may reduce the amount of TGF-β released by the PRP.[13] In another study it was shown that centrifugation speed of higher than 900 rpm (100g), can lead to platelet activation and resulting decrease of platelet reactivity.

Selective filtration technology or platelet apheresis depends on a single-use disposable 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) without the need for centrifugation. Compared to a commercial centrifuge-based method, the filtration device produces a blood fraction similarly enriched in platelets and growth factors.[14] Currently, only platelet collection using an apheresis machine enables these objectives to be easily achieved.[15,16]

Despite these variations, all protocols follow a generic sequence that consists of blood collection, an initial centrifugation to separate red blood cells, subsequent centrifugations to concentrate platelets, and other components and an activation of the sample by adding a platelet agonist (Figure 1). In addition to the platelets, it is important to analyze red and white blood cells in the preparation.

Platelet activation is reduced postprandially (5). However, a gentle mastication is able to induce the release of pro-inflammatory components into the bloodstream, especially when patients have severe periodontal disease (6). Thus, it is preferable that patient be fasting before preparing the PRP to reduce pro-inflammatory factors in platelet concentrate. In addition, aspirin, corticosteroids and NSAIDs affect platelet functions and should be avoided at least during 10 days before blood collection.

After blood collection, it is mandatory to prepare the PRP as soon as possible (ideally within 1 hour) to avoid undesired non-specific platelet activation. The PRP is stable for about 3 to 4 hours at room temperature, but platelets can become refractory to agonist stimulation.[17,18,19]

HANDLING AND APPLICATION OF PRP

Once the PRP is prepared, it is stable in the anti-coagulated state for eight hours or longer, permitting the blood to be drawn before operation and used as needed during lengthy procedures.[20] It must be activated for the platelets to release the contents of their α-granules, with the clot that forms providing a vehicle to contain the secreted proteins and maintain their presence at the site of application. This is most commonly accomplished by adding a solution of 1000 units of topical bovine thrombin per millilitre in 10% calcium chloride to the PRP.[36] Marx[37] described a technique in which 6 ml of PRP, 1 ml of the calcium chloride/ thrombin mix and 1 ml of air are introduced into a 10 ml syringe, with the air acting as a mixing bubble. The syringe is agitated for six to ten seconds to initiate clotting, and the clot is then delivered. Man, Plosker and Winland-Brown[38] described an alternative technique for delivering the activated PRP. The PRP and calcium chloride/ thrombin solution are mixed in a 10:1 ratio using a dual syringe system. The PRP is drawn into a 10 ml syringe and the activating solution is drawn into a 1 ml syringe. Both syringe plungers are connected to move in concert with both output ports connected to a dual spray applicator tip which allows both solutions to be mixed as they are applied to the surgical bed. Because α-granules immediately release their contents on activation, the clotted PRP should be used within ten minutes of clot initiation. This issue is circumvented in the dual syringe delivery system because PRP is

delivered to the wound site immediately after activation. In the case of other mixing techniques, it is important to transfer the clot to the surgical site before retraction, otherwise the clot that is transferred may be deficient in secretory proteins.

In contradiction to the above method of activation in some studies un-activated PRP is used with the belief that collagen is a natural activator of PRP, thus when PRP is used in soft tissue, it does not need to be exogenously activated.[39]

CELLULAR COMPOSITION AND BIOLOGICAL EFFECTS OF PRP

In spite of efforts to inject similar amounts of PRP to patients, there is a high variability in PRP obtained. There is limited information available regarding the optimal platelet and WBC content necessary to achieve a desired biologic effect and it may be that specific products are better for certain applications. Platelets increase anabolic signalling and, in contrast, leukocytes increase catabolic signalling molecules. Depending on how they are obtained and prepared, PRPs present highly variable concentrations of platelets, erythrocytes and leukocytes.[20,21,22] Previously, investigators have defined platelet-rich plasma according to platelet concentration. Marx defined platelet numbers of $1000x10^3/\mu l$ as therapeutic PRP[23] whereas Mazzucco et al., in 2009, has defined platelet numbers of $>200x10^3$ platelet/μL as sufficient for a therapeutic effect.[24] But Jungbluth et al, described a platelet concentration three to five times higher than in peripheral blood to be advantageous to stimulate bone regeneration. Below this concentration, they found the effect of PRP to be suboptimal, and paradoxically, a higher concentration was shown to inhibit bone regeneration.[25] Similarly, Giusti et al. showed that PRP preparation with $0.5\text{-}1x10^6$ platelet/μL induced proliferation, migration, collagen, and MMPs production when compared to untreated tenocytes in culture. Higher concentrations were found to have inhibitory effects on the proliferation, migration of tenocytes and overall production of collagen. In contrast, matrix metalloprotease production increased with increasing concentration of platelet, which could be counterproductive as excessive proteolysis, can impair tendon mechanical stability.[26] In general it has been suggested that for maximum efficacy, platelet concentration in PRP should be three to four times that of whole blood, i.e. between 600,000 and 900,000 platelets per microliter.[27,28,29]

But there is still no agreement on the concentration of leucocytes that should be present in therapeutic PRP. Proponents of PRP containing high white blood cell concentrations believe that the presence of WBC provides natural protection against infections and allergic responses. Other authors do not recommend the presence of high white blood cells concentration in PRP as they destroy surrounding tissue.[30] PRP containing concentrated leukocytes release more catabolic cytokines compared to PRP with low leukocytes.[31] Presence of high white blood cells concentration prevented proliferation of osteocytes and myocytes whereas it did not seem to have any effect on tenocyte proliferation.[32] Furthermore, platelets and white blood cells contribute to the formation of microaggregates in the red blood cells, which are thought to be deleterious for the recipient.[33] However, clinical positive effects of pure-PRP have not been demonstrated in controlled studies yet, and, in many clinical controlled studies, only a slight reduction of pain was obtained after a leukocyte rich-PRP injection. Moreover, it has been demonstrated that the anti-bacterial effect of PRP against Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes and meticillin-resistant Staphylococcus aureus was not linked to the presence of leukocytes.[34]

Healing of injured tendinous tissue is mediated by a complex array of intra- and extra-cellular events that are regulated by signaling proteins. This entire process is incompletely understood. Disruption of the vascular structure as a result of injury leads to the formation of fibrin and platelet aggregation.[35,36] A stable blood clot is then formed by coagulation of the blood. Subsequently, several growth factors are released

into the injured tissue from the platelets and other cells that induce and support healing and tissue formation.[37] PRP is also activated by the addition of thrombin and calcium, resulting in the release of a cascade of growth factors from the α-granules.[38] These granules contain numerous proteins which are members of the families of growth factors, cytokines and chemokines that provide a powerful influence on tissue healing. They include platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet-derived angiogenesis factor (PDAF), Vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), Connective tissue growth factor(CTGF) insulin-like growth factor (IGF), osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin-1 (Table 1). The interaction between these growth factors and surface receptors on the target cells activates the intracellular signaling pathways that induce the production of proteins needed for the regenerative processes such as cellular proliferation, matrix formation, osteoid production and collagen synthesis. Release of these growth factor and catabolic cytokine concentrations are influenced by the cellular composition of PRP.

Tendon tissue is known to heal slower than connective tissues because of a poor intrinsic vascularization. However, in a study done on 24 patients affected by jumper's knee who underwent US-guided PRP injection, intratendinous vascularity needed to improve tendon regeneration was significantly increased.[39]

IN VITRO AND ANIMAL STUDIES OF PRP ON TENDINOPATHIES

Unlike the variations seen on application of PRP to patients, *in vitro* using cell cultures and in animals have shown more consistent results which have helped elucidate the biological mechanism of healing promoted by PRP.

PRP prepared by different methods results in a preparation that secretes different amounts of growth factors which in turn could either inhibit or speed up the healing process. Different growth factors significantly increased type I and III collagen production in tendon fibroblasts. Tendons cultured in 100% PRP showed enhanced gene expression of the matrix molecules, with no concomitant increase in the catabolic molecules. Moreover, releases from both PRP and PPP clots stimulate tendon cell proliferation, in contrast to unclotted PPP. In studies of human tenocyte culture both PRP, and PPP, stimulate cell proliferation and total collagen production. However, only PRP but not PPP slightly increases the expression of matrix-degrading enzymes and endogenous growth factors.[40]

In an *in vitro* study, Aspenberg and Virchenko injected PRP percutaneously into transected tendo Achillis in the rat.[41] This increased the strength and stiffness of tendon callus by about 30% after one week. Mechanical testing indicated an improvement in maturation of the callus. Kajikawa et al[42] showed that PRP injected locally in the rat patellar tendon increased the activation of circulation-derived cells and the immuno-reactivity for types I and III collagen at an early phase of tendon healing. The osteo-inductive effect of PRP on tendon-to-bone healing was evaluated on repair of the infraspinatus in a sheep model using MRI and histological study.[43] The results showed increased formation of new bone and fibrocartilage at the healing site.

CLINICAL STUDIES OF PRP

Although the majority of clinical studies have yielded excellent outcomes, most are only limited to case reports or small series. The evidence of enhancement of tissue healing by PRP remains largely anecdotal. Very few clinical studies with prospective or retrospective controls have demonstrated a significant enhancement of healing of hard and soft tissue with the use of PRP.

One of the first clinical applications of PRP was the addition of autologous fibrin adhesive to cancellous bone during mandibular reconstruction. This study, published in 1994, described radiographic consolidation of bone after four weeks, as opposed to eight in controls which was

attributed to enhanced osteo-conduction given to the osteo-competent cells in the graft by the fibrin network developed by the concentrated platelets.[44]

Buffered PRP has been used as an alternative to surgery in patients with lateral epicondylitis who had not responded to conservative treatment. In Mishra's series, 15 patients showed significant improvement with a single injection, and this was sustained over time with no reported complications.[45]

In another study, Sánchez et al investigated the effect of PRP in ruptures of the tendo- Achillis in athletes who underwent open repair. The procedure was undertaken in conjunction with a preparation rich in growth factors (PRGF) in six athletes and compared retrospectively with a matched group who had the conventional surgical procedure. Those receiving PRGF recovered their range of movement, showed no wound complications and took less time to resume training.[46]

The potential of using PRP in repair of the rotator cuff was evaluated in a pilot study by Randelli et al.[47] After repair of the tear, 14 patients received intra-operative autologous PRP combined with an autologous thrombin component. They were followed up for 24 months and demonstrated a significant reduction in their pain score and significant increases in functional scoring. In another study of 33 patients, there was some evidence for small short-term symptomatic improvements with the addition of autologous blood injection to standard treatment for Achilles tendinopathy.[48]

Other authors have shown that in patients with chronic patellar tendinopathy (jumper's knee), PRP-treated group demonstrated significantly greater improvements compared with focused extracorporeal shock-wave therapy (ESWT).[49,50]

Question about how many PRP injections are needed remains unanswered. To date, no consensus on the minimal number of PRP injections that optimizes tendon healing has been published. Most growth factors contained in platelets are short-lived and thus, repeated administration is advised. Patellar tendinopathy is a common disorder that can affect athletes in different sports at all levels of activity. Dragoo et al. in a randomized controlled study have proved that a single injection of PRP was enough to accelerate the recovery from patellar tendinopathy relative to exercise and ultrasound-guided dry needling alone, but they remarked that the benefit of PRP dissipates over time.[51] Zayni et al treated 40 athletes with proximal patellar tendinopathy over a period of three years and showed that two consecutive PRP injections in chronic patellar tendinopathy showed better improvement in outcomes when compared to single injection.[52] Charousset et al. studied a series of 28 athletes and demonstrated that the application of 3 consecutive US-guided PRP injections significantly improved the symptoms and function in athletes with chronic PT and allowed a more rapid recovery to their pre-symptom level of sporting participation. In addition, they found a return to a normal architecture of the patellar tendon after this treatment on MRI assessment.[53] Other clinical studies suggest that a weekly repeated injection of PRP permitted better clinical outcomes. Filardo et al. treated 15 patients with multiple PRP injections and observed a statistically significant improvement in knee function and quality of life, and most patients had a good recovery and returned to their previous sporting activity level.[54]

However, de Vos et al did not find any greater improvement in pain and activity in 54 patients with chronic Achilles tendinopathy who were treated with eccentric exercises, a PRP injection or with a saline injection. No enhanced tendon structure and neovascularisation was observed in the PRP treated athletes compared to the placebo.[55,56,57] Similarly, PRP injection was found to be no more effective in improving quality of life, pain, disability, and shoulder range of motion than placebo in patients with chronic rotator cuff tendinopathy who were treated with an exercise program.[58] MRI appearance of diseased Achilles tendons also remained largely unchanged following

PRP injection.[59] Similarly, Bell et al showed that the administration of two unguided peritendinous autologous blood injections one month apart, in addition to a standardized eccentric training program, provided no additional benefit in the treatment of mid-portion Achilles tendinopathy.[60]

POTENTIAL RISKS OF PRP USE

Because PRP is prepared from autologous blood it is inherently safe, and any concerns regarding transmission of diseases such as HIV, hepatitis, or of immunogenic reactions that exist with preparations of allograft or xenograft, are eliminated. However, the activation of PRP involves using calcium chloride and bovine thrombin preparations, which contain bovine factor V. 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.[61] The bovine thrombin preparations used in these cases were of high dose (> 10,000 units) and were applied directly to open wounds, where absorption into the systemic circulation is certain. There have been no similar reports since 1997 owing to the use of highly purified bovine thrombin. 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 does not produce anti-factor V antibodies.

CONCLUSION

In spite of the growing clinical use of PRP, there is still no standardized method of producing PRP for clinical use which is universally acceptable. This creates difficulties in comparing the results of PRP and precludes establishment of standards for basic and clinical studies. The clinical use of PRP for a wide variety of applications has been described, particularly in periodontal, craniofacial and orthopedic spinal surgery. However mostly the evidence is anecdotal and inconclusive as most studies do not include controls. The available scientific evidence does not warrant the use of PRP for the first-line treatment of tendinopathies. PRP therapy may, however, deserve consideration in specific tendinopathies subtypes, after failure of ultrasound-guided corticosteroid injections. Nevertheless, further studies are needed to define these potential indications and the optimal treatment protocols. It is important to note that the complexity of the tendon healing process cannot be replicated simply by injecting a subset of growth factors, whose effects may occur in opposite directions over time.[3] Additionally, there is also significant variation in platelets and white blood cells with repetitive blood draws from the same individual at different times for all methods of platelet-rich plasma separation. Considering platelet-rich plasma application methods tend toward a repetitive treatment, the lack of consistency at the level of the individual is important, as reliable platelet-rich plasma dosages may be needed to produce consistent results.[62] Improving the techniques for obtaining PRP is crucial, as the injection protocol. Finally, post-infiltration rehabilitation remains absolutely necessary.

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