The Evolving Role of Platelet Rich Plasma in Tendinopathies

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Abstract

Tendinopathies are chronic affections of the attachments of muscles to the bones. These have traditionally been treated conservatively by activity restriction, non-steroidal anti-inflammatory drugs, physical therapy and judicious use of orthotics. Unresponsive patients are being treated by locally acting steroid injections with varied results. Surgical options are used sparingly when indicated. Platelet rich plasma has been tried by various researchers with the aim of a biological cure with minimal side effects and has shown promising results. The objective of this article is to describe the biological role of PRP, elucidate the current various techniques of PRP production and to evaluate the results of the use of PRP in tendinopathies.

Platelet rich plasma (PRP) is a novel method of treatment for tendinopathies and is a current area of interest for clinicians and researchers. The growth factors and the mechanisms involved in the biology of healing of Tendinopathies due to PRP are still incompletely understood. Many researchers have shown rapid tissue healing with PRP [1], which is promising. PRP is easy to procure and is cost effective. Since PRP is derived autologously from the patient’s own blood there are no concerns about immunological reactions and disease transmission associated with other allergic biological treatments like fibrin glue. Recombinant tissue growth factors developed by bio-engineering have short shelf lives and are expensive and these two disadvantages has precluded their wide spread use in routine clinical practice.

Platelet activation releases factors like platelet derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial Growth factor (VEGF), Epidermal growth factor (EGF), Fibroblast growth factor (FGF), connective tissue growth factor (CTGF) and Insulin like growth factor (IGF), which cause tissue healing by promoting cellular chemo taxis, proliferation and differentiation of cells, removal of cellular debris, vascular invasion by angiogenesis and formation of extracellular matrix [2].

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 did not include controls. There is still no standardized method of producing PRP for clinical use which is universally acceptable which creates difficulties in comparing the results of PRP and precludes establishment of standards for basic and clinical studies.

The level of evidence remains low, as few well-designed randomized controlled trials have been published. The available scientific evidence does not warrant the use of PRP for the first-line treatment of tendinopathies. PRP therapy may deserve considerations in specific tendinopathy subtypes or after failure of ultrasound-guided corticosteroid injections. Nevertheless, further studies are needed to define these potential indications and the optimal treatment protocols. A key point is 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].

The objective of this article is to review the current information 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 the abstracts were analyzed. Articles with links of full access were downloaded and abstracts relevant to the research question were also downloaded.

Preparing PRP

Platelets are cytoplasmic fragments of megakaryocytes, a type of white blood cell, and are formed in the bone marrow. They are the smallest of the blood cells, round or oval in shape and approximately 2 μm in diameter. They lack nuclei but contain organelles and structures such as mitochondria, microtubules, and granules (α, δ, λ). The α

Granules, bound by a membrane, are formed during maturation of the megakaryocytes. They are about 200 nm to 500 nm in diameter, and number approximately 50 to 80 per formed platelet [4]. They contain more than 30 bioactive proteins,
many of which have a fundamental role in haemostasis or tissue healing. Haemostasis can be considered to be the first stage of healing of injured tissue [5].

Platelet-rich plasma (PRP), also known as platelet-enriched plasma (PePR), 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 [6]. Result variability in clinical and experimental studies may be explained by a poor knowledge about PRPs [7]. Depending on how they are obtained and prepared, PRPs (or Platelet-Released Growth Factors PRGF) present highly variable concentrations of platelets [8-10], erythrocytes and leukocytes. It is still necessary to optimize the techniques for obtaining and concentrating PRPs and standardizing the injection. Ideally, platelet concentration should be three to four times that of whole blood, i.e. between 600,000 and 900,000 platelets per microlitre [11-13]; a concentration higher than 1,200,000 platelets may, indeed, be unfavorable [14]. A concentration of 1.407 × 10^10 to 3.201 × 10^10 platelets in plasma has been suggested to be the working definition of PRP [15]. This is a platelet count five times higher than that of the blood, which is normally 150,000/μl to 350,000/μl, with an approximate mean of 200,000/μl. PRP refers to autologous preparations. However, Anitua et al. [16], stated that the platelet count of PRP should be just above 300,000/μl. On the other hand, Choi et al. [17], showed that high PRP concentrations (>10%) suppressed, but that low PRP concentrations (1% to 5%) stimulated, the viability and proliferation of alveolar bone cells. Thereby, supporting the view that variations in PRP concentrations may lead to variable effect on tissue regeneration. Ideally, a reproducible PRP (with identical platelet concentration) should be injected to all patients.

There are three techniques for preparation: 1. the gravitational platelet sequestration (GPS) technique, 2. Standard cell separators and 3. Autologous selective filtration technology (plateletpheresis). The GPS is a table-top centrifuge system. 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 PRP yield is approximately 10% of the volume of whole blood drawn. This system used a flat-bottomed, 60-ml plastic centrifuge tube. The PRP volume of about 5 ml can be collected following a 12-minute spin at 3200 rpm. 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 and both a hard (fast) and a soft (slow) spin, yielding platelet concentrations from two to four times baseline. Weirich and Kleis [18] described a discontinuous technique with a cell separator that produces a fivefold increase in platelet count. The red blood cells and some, or all, of the platelet-poor plasma (PPP) can be returned 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 eightfold [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.

Centrifugation must be sterile and precisely suited to separating platelets from red blood cells with adequate concentrations of platelets [19]. Not all currently available commercial devices may be the 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 [5]. 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 [21].

Selective filtration technology or platelet aphaeresis 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 [22].

Currently, only platelet collection using an aphaeresis machine enables these objectives to be easily achieved [23, 24]. The presence of white or red blood cells could be adverse to tissue healing process. It was demonstrated in vitro and animal studies that their absence limits the inflammatory response [25]. 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 methicillin-resistant Staphylococcus aureus was not linked to the presence of leukocytes [26].

Finally, platelet activation is reduced postprandially [27]. Moreover, a gentle mastication is able to induce the release of pro-inflammatory components into the bloodstream, especially when patients have severe periodontal disease [28]. Thus, it would be preferable that patient should 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.

Centrifugation speed should be set to a maximum of 900 rpm (100g), since a higher speed can lead to platelet activation and resulting decrease of platelet reactivity.

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 [29,31]. Usually, citrate [or 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.

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, [27] with more than 95% of the pre-synthesized growth factors secreted within one hour [28]. After the initial burst of growth factors, the platelets synthesize and secrete additional such factors for the remaining several days of their life span.

**Biological effects of PRP**

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 [32,33]. 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 [34]. 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 [35]. These granules contain numerous proteins which are members of the families of growth factors, cytokines and chemokine 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.

![Figure 1](image_url)
ten minutes of clot initiation. This is not a problem with the contents on activation, The clotted PRP should be used within the surgical bed. Because the α granules quickly release their which allows both solutions to be mixed as they are applied to with both output ports connected to a dual spray applicator tip syringe. Both syringe plungers are connected to move in concert 10 ml syringe and the activating solution is drawn into a 1 ml PRP and calcium chloride/thrombin solution are mixed in a an alternative technique for delivering the activated PRP. The is agitated for six to ten seconds to initiate clotting, and the clot before operation and used as needed during lengthy procedures [10]. 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 milliliter of 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 contains both PRP, and PPP, stimulate cell proliferation and total collagen production. In studies of human tenocyte culture both PRP and PPP clots stimulate tendon cell proliferation, in contrast to unclotted PPP. In 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].

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 [10]. 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 milliliter of 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 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 the α granules quickly release their contents on activation, The clotted PRP should be used within ten minutes of clot initiation. This is not a problem with the dual syringe delivery because the 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 the 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].

In vitro and animal studies of PRP

The effect of PRP on regeneration has been studied on damaged tendon modules [40] All three TGF-β isoforms 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. PRP but not PPP slightly increases the expression of matrix-degrading enzymes and endogenous growth factors [41].

The positive effect of PRP on tendon healing has been established in several studies. In an in vitro study, Aspenberg

| Table 1: Various growth factors and their actions. |
| --- | --- | --- | --- |
| S. No | Platelet Growth factor Type | Growth factor Source | Biological Actions |
| 1 | Platelet derived growth factor (α-β) | Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells | Mitogenic for mesenchymal cells osteoblasts, stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells, regulates collagenase secretion and collagen synthesis, stimulate macrophage and neutrophil chemotaxis |
| 2 | Transforming growth factor TGF (α-β) | Platelets, extracellular matrix of bone, cartilage matrix, activated TH1 cells and nature killer cells, macrophages/monocytes and neutrophils | Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of growth factors, stimulates endothelial chemotaxis and angiogenesis, inhibits macrophage and lymphocyte proliferation |
| 3 | Vascular endothelial growth factor, VEGF | Platelets, endothelial cells | Increase angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells |
| 4 | Epidermal growth factor, EGF | Platelets, macrophages, monocytes | Stimulates endothelial chemotaxis / angiogenesis, regulates collagenase secretion, stimulates epithelial / mesenchymal mitogenesis |
| 5 | Fibroblast growth factor, FGF | Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts | Promotes growth and differentiation of chondrocytes and osteoblasts, mitogenic for mesenchymal cells, chondrocytes and osteoblasts |
| 6 | Connective tissue growth factor CTGF | Platelets through endocytosis from extracellular environment in bone marrow | Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion |
| 7 | Insulin like growth factor -1 IGF | Plasma, epithelial cells, endothelial cells, fibroblasts, smooth muscle cells, osteoblasts, bone matrix | Chemotaxis for fibroblasts and stimulates protein synthesis, enhances bone formation by proliferation and differentiation of osteoblasts |

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and Virchenko [42] injected PRP percutaneously into transected tendon Achilles in the rat. 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. Kajiwaka et al [43] showed that PRP injected locally in the rat patellar tendon increased the activation of circulation-derived cells and the immuno-reactivity for type 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 [44]. The results showed increased formation of new bone and fibrocartilage at the healing site.

Clinical studies of PRP

In clinical studies, details of the quantity of PRP used and the methods of application are procedure specific. Although the majority of these studies have yielded excellent outcomes, most are only limited case studies or small series. This evidence of enhancement of tissue healing by PRP remains largely anecdotal. A small collection of 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 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 [45].

The clinical reports are predominantly case studies or limited case series. No published level-1 clinical data supporting the use of PRP is available till date.

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, [46], 15 patients showed significant improvement with a single injection, and this was sustained over time with no reported complications.

In a case-control study, Sánchez et al [47] investigated the effect of PRP in ruptures of the tendo- Achilles 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.

The potential of using PRP in repair of the rotator cuff was evaluated in a pilot study by Randelli et al. [48]. 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.

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 [49]. 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

Based on the inconclusive results in the literature, this review cannot provide solid evidence in favor of the application of PRP in trauma and Orthopaedic surgery. However, because the majority of the clinical trials have shown encouraging outcomes, further multi-centric controlled clinical trials to elucidate the beneficial effects of PRP with uniform protocols in preparation are warranted before PRP can become part of standard treatment of tendinopathies. 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. Improving the techniques for obtaining PRP is crucial, as the injection protocol. Finally, post-infiltration rehabilitation time remains absolutely necessary.

REFERENCES


