Treatment of Tendon and Muscle Using Platelet-Rich Plasma

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Musculoskeletal injuries and impairments result in over 100 million office visits in the United States per year. Tendons and muscle-related issues account for a significant percentage of these visits. As our population ages and remains active, the number of orthopedic-related problems will rise dramatically.[1] Younger and older patients expect faster recovery from their injuries with less invasive procedures. Within this landscape, PRP has become a potential standalone or adjunctive treatment.

The concept of using the growth factors within PRP to help heal wounds dates back to the early 1980s.[2] Its use in orthopedic surgery, however, began during this decade and initially focused on the augmentation of bone grafting. The efficacy of PRP to accelerate bone healing continues to be debated in the literature.[3–8] Employing PRP to augment tendon healing, however, has been advocated only recently.[9,10]

PLATELET-RICH PLASMA BIOCHEMISTRY

PRP is a bioactive component of whole blood. The specific elements of PRP have not been uniformly defined in the literature. PRP, in general, has a higher concentration of platelets compared with baseline blood. Clinically valuable PRP, however, typically contains 1 million platelets or more per microliter.[11] Some authors define PRP as only platelets whereas others note that PRP may also have increased concentrations of white blood cells. The white blood cells within some forms of PRP contain important cytokines and enzymes. For example, Horsburgh and colleagues[12] found that platelet-derived mediators may be responsible for increased monocyte adherence in vitro. This adherence may be important for long-term tissue regeneration that is macrophage

mediated. Importantly, in vitro studies have also found that PRP significantly inhibits the growth of *Staphylococcus aureus* and *Escherichia coli*. In one of these studies, PRP was found to have no activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, or *Enterococcus faecalis*.[13,14]

PRP activation and the pH of PRP are other parameters that are being debated in the literature. Thrombin and calcium have historically been used to activate platelets. This combination results in the formation of a gel that may be used in open surgery but cannot be injected even through a large-gauge needle. Thrombin and calcium activation results in rapid release of contents of the granules within platelets. This requires immediate use of the PRP. Platelets, however, can be slowly activated by exposure to tendon-derived collagen.[15] This can produce in vivo activation and allows for administration of PRP through a small-gauge needle. Variations in partial activation with calcium are also being explored.[16] Liu and colleagues[17] have also found the release of growth factors from PRP to be pH dependent.

When platelets are activated either ex vivo or in vivo, they release the growth factors and proteins that reside within their alpha and dense granules. The alpha granules contain cytokines including platelet-derived growth factor, transforming growth factor-β, and vascular endothelial growth factor, among many others (**Table 1**).[18] Concentrations of these growth factors rise linearly with increasing platelet concentration.[11,19] After release, the cytokines are free to bind to transmembrane receptors on the surface of local or circulating cells. They then initiate intracellular signaling, which results in the expression of proteins responsible for cellular chemotaxis, matrix synthesis, and proliferation. Tissue regeneration through angiogenesis, extracellular matrix production, and collagen synthesis is orchestrated by the autocrine and paracrine effects of the growth factors. Everts and colleagues[20] have elegantly outlined the electron micrographic properties of how PRP releases these proteins. Properly prepared PRP in an unactivated form clearly reveals an abundance of platelets in a photomicrograph at high power (**Fig. 1**).[21]

Much emphasis has been placed on alpha granules but dense granules also play a role in tissue modulation and regeneration. The dense granules contain adenosine, serotonin, histamine, and calcium.

Adenosine is a nucleoside that plays an important role in many biochemical processes, including transfer of energy. Adenosine is a primary cytoprotective agent that prevents tissue damage. Adenosine receptor activation has been shown to have an anti-inflammatory effect during the inflammatory process associated with diabetic nephropathy.[23] In laboratory studies, adenosine A2A receptor agonists applied topically to diabetic foot wounds have been effective in tissue repair and reconstruction and their effect on difficult wounds in humans is currently under investigation.[24] Adenosine also has the ability to increase IL-10 production by macrophages in some cases.[25] This increase in IL-10 could indicate a change in the character of the

Table 1		
Selected growth factors within platelet-rich plasma[19,22]		
Growth Factor	**Actions**	
Platelet-derived growth factor	Chemoattractive for mesenchymal stem cells and monocytes	
Transforming growth factor-β	Mitogen for fibroblasts and enhances extracellular matrix production	
Vascular endothelial growth factor	Stimulates angiogenesis	
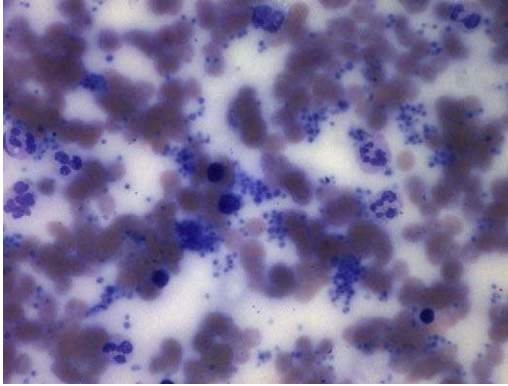
Fig. 1. Photomicrograph of PRP. Note high concentration of platelets.

macrophage to an anti-inflammatory state. In other instances, it appears that adenosine can function to activate macrophages to produce pro-inflammatory cytokines, IL-1 and IL-18.[26]

Serotonin is a monoamine neurotransmitter. This hormone can be exponentially more effective at increasing capillary permeability than even histamine.[27] Serotonin also acts as a chemoattractant for fibroblasts and increases their proliferation. Interestingly, macrophage cells have receptors that are sensitive to serotonin. Serotonin injected locally into tissue induces an influx of macrophages into that tissue.[28] Serotonin has also been shown to effect macrophages by suppression of IFN–gamma-induced 1a expression at sites of inflammation.[29] These data suggest strong interactions between serotonin and macrophages. This relationship and the particular effect serotonin has on cellular interactions should be considered when evaluating the effects of PRP on inflammation and healing.

Histamine is a biogenic amine involved in local immune responses. Locally, it also acts as a vasodilator. Histamine enhances permeability of the microvascular system of capillaries and venules. This increased permeability is due to the contraction of endothelial cells and removal of fenestrated diaphragms blocking gaps in the endothelial lining.[30] At the time of injury, histamine is released, acting as a vasodilator that actively increases endothelial membrane permeability. This increase in membrane permeability allows inflammatory and immune cells greater access to marginate and enter the local area. Histamine is also a strong activator of macrophages.

Calcium is the final component of the dense granules. Involvement of calcium in wound healing is mainly in keratinocyte proliferation and differentiation. Skin fibroblasts require calcium but are far less sensitive than keratinocytes to its effects. Calcium may also be required in epidermal cell migration and regeneration in the remodeling phase. Although calcium dressings are meant to serve in the hemostatic phase of healing, it is unclear whether their effect carries over into the later remodeling phase. The effect of calcium is essential in wound management, and the calcium content within the dense granules of platelets may play a vital role in its delivery to the site of injury.[31]

The unique combination and concentration of bioactive molecules that exist within PRP have profound effects on the inflammatory, proliferative, and remodeling phases of wound healing. Researchers worldwide are evaluating how PRP produces these effects. Not all cytokines within PRP have been characterized. These cytokines also exist in hyperphysiologic concentrations in PRP when compared with whole blood.

Since healing of tendon and muscle is similar to wound healing in some respects, PRP has great potential to improve soft tissue healing. The concept of using PRP to restore tendons and muscle after injuries is explored in this context.

TENDON INJURIES AND HEALING

Tendon injuries and disorders come in many forms (**Table 2**). The generic term, tendinopathy, is best used to describe these many forms. The spectrum of problems ranges from acute tendonitis to chronic tendinosis to full-thickness tearing. Extrinsic factors, for example, a hooked acromion in the shoulder, combined with intrinsic factors, such as age-related degeneration, can contribute to tendinopathy. Repetitive microtrauma or exposure to fluoroquinolone antibiotics has also been implicated.[32,33] Genetic factors, matrix metalloproteases (MMPs), and apoptosis may further contribute to tendon degeneration.[34]

Tendon healing occurs through 3 phases: inflammation, proliferation, and remodeling. These overlapping phases are controlled by a variety of growth factors. They are also linked through complex cellular signaling cascades.[22,36] For example, the temporal expression of growth factors has been reported to be important in supraspinatus tendon healing.[37] Since PRP contains many of these cytokines and cells in hyperphysiologic doses, it may be a reasonable choice to help initiate or accelerate tendon healing. The use of PRP for tendon disorders is presently being investigated for significant tendon disorders, such as chronic severe tendinosis, or in combination with surgery for complete tendon tears.

Use of PRP in Tendinopathy

In vitro studies have found that PRP can enhance human stromal and mesenchymal stem cell proliferation.[38] Conversely, Woodall and colleagues[39,40] found that PRP suppresses macrophage proliferation and IL-1 production within the first 72 hours after exposure. This differential induction of cells has important implications for tendon and muscle healing. It may be possible for PRP to initially inhibit excess inflammation while stimulating proliferation and maturation. This may be especially important in preventing the fibrous scar tissue healing that occurs with macrophage-mediated tendon-to-bone healing.[41] Future studies should evaluate the possibility that PRP may also stimulate tendon stem cells that have recently been identified.[42]

Equine and human cell culture studies support the use of PRP for the treatment of tendon injuries and disorders. Schnabel and colleagues[43] reported enhanced type I

Table 2 Types of tendon problems	
Type of Tendon Problem[35,36]	Findings
True tendonitis	Associated with acute increase in activity, eg, patellar tendonitis with hill running
Tendinosis	Common, misdiagnosed as "tendonitis." A chronic degeneration of a tendon, eg, "tennis elbow"
Torn tendon	Common, can occur with trauma or spontaneously through chronic tendinosis, eg, Achilles tear or rotator cuff tear
Tendinopathy	Generic term for tendon disorder
Tendon-related pain	What the patient complains of and what the clinician needs to treat

collagen gene expression in PRP-cultured tendon cells, with no concomitant increase in catabolic molecules, such as matrix metalloproteinase 3 (MMP-3). Other authors, however, have found that PRP not only stimulates human tenocyte proliferation and total collagen production but also slightly increases MMP-3 expression.[44] Anitua and colleagues[45] reported that the balance between TGF-β and other secreted cytokines may control angiogenesis and fibrosis.

Aspenberg and Virchenko reported greater maturation in tendon callus when PRP was used to augment rat Achilles tendon tears. They also reported increased force to failure and ultimate stress in PRP-treated animals.[46,47] In a landmark study, Kajikawa and colleagues[48] found that PRP enhances the mobilization of circulation-derived cells to an area of injection. They also found that PRP induced type I collagen production and increased the proliferation of macrophages at 3 and 7 days. This article did not, however, measure macrophage proliferation in the first 48 hours, so it is not possible to directly compare it to the work of Woodall et al, which showed initial macrophage suppression during that period.

Mishra and Pavelko were the first to report the use of PRP for patients considering surgery for chronic severe elbow tendinosis (**Fig. 2**).[9] All of the patients had failed a standardized nonoperative treatment protocol. In this prospective, controlled pilot study using unactivated and buffered PRP, the authors found 60% improvement in pain scores for PRP-treated patients versus a 16% improvement in control patients 8 weeks after treatment. This was a small, nonrandomized study. At final follow-up (mean, 25.6 months; range, 12–38 months), however, the PRP patients reported over 90% reduction in pain compared with pre-treatment scores. Ninety-three percent of patients were also fully satisfied with the treatment. A double-blind prospective randomized trial of 230 patients using this protocol in the United States has been initiated. No significant complications or worsening of symptoms has been reported using this technique.

Anitua and colleagues[45] found faster recovery in athletes undergoing PRP-enhanced Achilles tendon repair. In their study, athletes treated with surgery and PRP were compared with a retrospective control group of athletes treated with surgery alone. The PRP patients recovered range of motion earlier, had no wound complications, and returned to training activities in less time than control patients. The cross-sectional area of the PRP-treated tendons was also smaller than that in nontreated tendons when measured by ultrasound. Randelli and colleagues[49] recently

Fig. 2. Injection of PRP for chronic elbow tendinosis.

reported a case series of patients treated with PRP-augmented arthroscopic rotator cuff repairs. They found the technique to be safe without any reported complications and all patients recovered full passive range of motion within 1 month post-treatment. Gamradt and colleagues[50] reported on another technique for potentially enhancing rotator cuff repair with a different form of PRP. This method is presently being evaluated in a prospective, randomized trial.

Several other trials are underway in the United States and Europe to clarify the value of PRP for tendon injuries. Gosen and colleagues[51] are using unactivated PRP in a prospective double-blind randomized controlled trial of 100 patients to test PRP against cortisone in patients with chronic lateral epicondylar tendinosis. Preliminary data from their study find PRP patients demonstrating more reduction in pain and higher DASH scores at 24 weeks. Aspenberg and colleagues are presently conducting a prospective, randomized trial of PRP-augmented Achilles tendon repairs in humans. They will also be able to report biomechanical data because they are implanting tantulum balls above and below the repair site. This will allow measurement of tendon elongation postoperatively. Similar findings in the United States and Europe also support the use of PRP in the treatment of chronic Achilles tendinopathy (**Figs. 3** and **4**). (Mishra, personal communication, June 2008).

The Role of PRP in Muscle Injuries

Muscle injuries may be caused by a contusion by way of a direct blow, a strain, or occasionally a laceration. Rapid eccentric contraction is responsible for many of these injuries and the musculotendinous junction is the most common location of injury. Contact, sprinting, and jumping sports yield the most muscle injuries.[52] Although imaging studies may be included in the workup, diagnosis is based largely on patient history and physical examination. While there is no universal classification system for muscle injuries, the most common one has been adapted from Ryan's system (**Table 3**).[53]

Muscle healing, like tendon healing, occurs in a series of overlapping phases, including inflammation, proliferation, and remodeling. These events are also

Fig. 3. PRP injection for chronic Achilles tendinopathy.
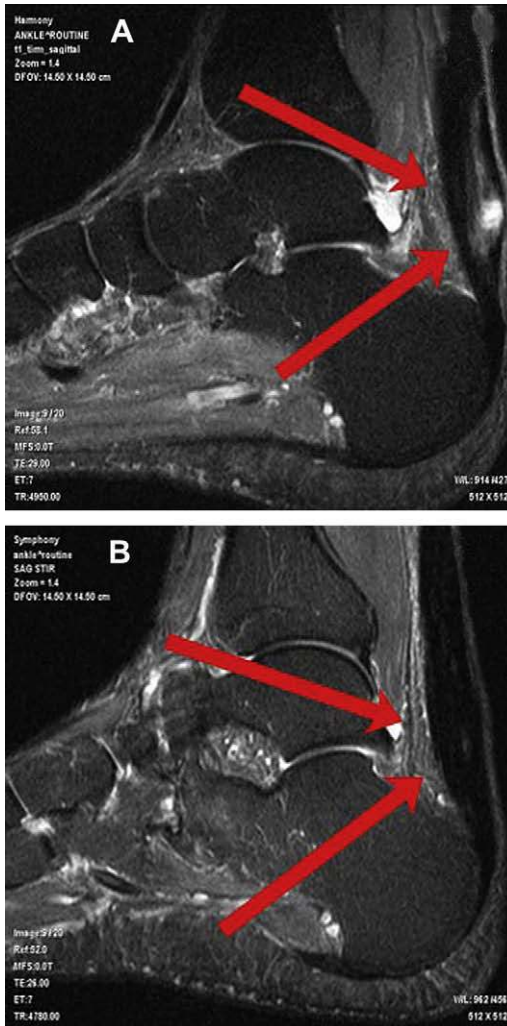
Fig. 4. Achilles MRI before and after PRP treatment. (*A*) MRI before PRP injection, partial Achilles tendon tear. (*B*) MRI 4 mo after PRP, healing of partial tear.

coordinated by growth factors and cell-to-cell interactions. Healing is dependent on local vascularity and regeneration of intramuscular nerve branches, both of which may be enhanced by PRP.[54,55] The speed of progression through these phases of healing depends on the severity of the injury and the efficiency of the patient's own biology in combination with any prescribed therapy and rehabilitation.

Despite the significance of this type of injury there are few clinical studies evaluating treatment options. Standard treatment plans attempt to decrease the bleeding and swelling associated with the injury. Recommendations include rest from activity, immediate application of ice, compressive dressings, and elevation of the affected limb. Administration of anti-inflammatory medications may alleviate pain; however, there is some evidence that this may interfere with the ability of the muscle tissue to heal. Nonsteroidal anti-inflammatory drugs may inhibit fusion of myogenic precursor

Table 3
Muscle injury grades

Grade	Tissue Damage	Symptoms
Grade 1	Few muscle fibers involved	Not apparent until conclusion of activity; very little swelling and pain only with activity
Grade 2	Moderate number of fibers involved with intact fascia	Immediately painful and moderately sore to palpation
Grade 3	Many fibers involved with incomplete fascial injury	Immediately painful and sore to palpation; patient may limp to avoid pain, severe pain with flexion vs. resistance and/or full extension
Grade 4	Complete dissociation of fibers and fascia; complete rupture	Immediate severe pain; ecchymosis below area; palpable defect

cells, thus impairing muscle healing.[56] Rehabilitation often involves a gradual return of the injured muscle to resistance exercise after the inflammatory phase has subsided. The ideal treatment for muscle injuries would accelerate the process of muscle healing while enhancing the quality of repaired tissue. The role of several growth factors in the natural repair of injured muscle is evident based on increased levels of these cytokines found in healing muscle tissue. PRP is known to contain many of these bioactive proteins.

The Role of PRP in Muscle Healing

Several growth factors within PRP have been evaluated in muscle healing. In vitro results investigating individual growth factors on skeletal muscle are variable, but certain growth factors are capable of enhancement of muscle regeneration and improved muscle force after injury.[57] Growth factors along with macrophages and the products of the COX-2 pathway regulate the inflammatory phase of skeletal muscle healing. Transforming growth factor-β1 and PGE2 may also function synergistically to balance the level of fibrosis during skeletal muscle healing.[58] In a mouse model of muscle laceration, insulin-like growth factor 1 and fibroblast growth factor-β improved muscle healing and increased fast-twitch and tetanus strength compared with controls at 1 month.[59] Autologous platelet concentrate used to treat muscle injury in a rat gastrocnemius contusion model resulted in increased satellite cell activation and myofibril width.[60] Acceleration of functional restoration was found in a human trial of elite athletes injected with ultrasound-guided PRP following muscle injury. These high-level athletes returned to sport at full strength in as early as half the expected recovery time without any evidence of excess fibrosis.[61] There are, however, no randomized controlled human studies supporting the use of PRP for muscle injuries. This is clearly an area that needs further in vitro and in vivo investigation. A prospective randomized trial using ultrasound-guided PRP for Grade 3 or Grade 4 injuries in elite athletes with return to play as an end point would provide helpful information.

DISCUSSION AND FUTURE CONSIDERATIONS

Athletes of all types are presently dissatisfied with their treatment options for tendon and muscle injuries. They are requesting better and less invasive methods to enhance or accelerate healing. Biologic options include the use of stem cells, gene therapy, and autologous or bioengineered cytokines. However, all of these possibilities are

currently experimental and are not available for clinical use. Growth factors, in the form of PRP, meet many of the criteria for the ideal biologic treatment. PRP is made from the patient's own blood, which makes rejection or an adverse reaction unlikely. It can also be prepared immediately at the point of care, which makes it simple and less expensive than stem cell therapies, which often require a period of sorting and culturing before clinical use.

The exact mechanisms by which PRP initiates cellular and tissue changes are presently being investigated. It is clear that PRP induces proliferation of a variety of cell types.[38,62] PRP has also been found to recruit reparative cells.[48] This helps explain why a single PRP application can have a lasting effect on the healing process. Through interaction with macrophages, PRP may control the inflammatory reaction and thus improve tissue healing and regeneration. It is clear from in vitro studies that PRP initially inhibits IL-1 production from macrophages and reduces their proliferation. By day 4, however, this inhibition turns to stimulation of IL-1 and macrophage division.[39] This initial suppression of macrophage activity may prevent the excessive early inflammation that can lead to dense scar tissue formation. It may further be possible for PRP to regenerate tissue phenotypically closer to normal tendon and muscle by stimulating quiescent stem cells.[42] This has yet to evaluated but should be investigated. Finally, investigating specific gene expression patterns in vitro and in vivo will contribute to a more detailed understanding of the mechanism of action of PRP.

The foregoing hypothesis supports the following one of how PRP may regenerate tendon or muscle function. PRP is applied in an unactivated form that becomes activated by the collagen within connective tissue. The PRP then releases its growth factors and cytokines. These bioactive proteins in turn stimulate local stem cells and enhance extracellular matrix gene expression. Recruitment of reparative cells from the local circulation or bone marrow then occurs. Simultaneously, PRP inhibits excess inflammation, apoptosis, and metalloproteinase activity. These interactive pathways may result in the restoration of tendon or muscle tissue, which can withstand loading with work or sports activity, thereby diminishing pain. PRP may also modulate the microvascular environment or alter efferent or afferent neural receptors. Much more investigation is required to verify the mechanism(s) of action of PRP.

Clinical investigation of PRP for tendon and muscle injuries and disorders is just beginning. There are only a few small, nonrandomized trials supporting the use of PRP for tendinosis or acute tendon tears.[9,10] Virtually no published evidence supports the use of PRP for muscle injuries in human clinical trials. Basic science data, however, point to a theoretical value. Fortunately, several prospective, double-blind randomized trials have been initiated for both tendon and muscle injuries. The results of these trials will guide future treatment recommendations.

As we look forward to these trials, it will be important to evaluate the inclusion and exclusion criteria rigorously. Defining the best types of tendon and muscle problems to treat with PRP will be a difficult but important task. The anatomic location of the injury may also be salient. For example, tendons have 3 distinct zones: the myotendinous junction, the midsubstance, and the osseotendinous junction. PRP most likely affects these zones differently. This has yet to be studied. The dosage and type of PRP employed, clearly, will also be critical elements for further study. Presently, there are proprietary PRP formulations and equipment to produce it. Standardized dosing and composition will be required to compare results. In addition, the value of ultrasound or other guidance mechanisms for injection need to be investigated. Finally, post-procedure protocols and rehabilitation methods must be coordinated to produce the best overall outcomes. For example, it may be better to gently load the tendon in the first few weeks to enhance healing.[63]

The tendon injuries that may be improved using PRP include, but are not limited to, repairs of Achilles, patellar, quadriceps, or rotator cuff tendon tears. Chronic tendinosis of any tendon may also benefit. Specifically, it could be possible to treat an acute Achilles tendon tear nonoperatively using a PRP injection. Careful evaluation will be required to determine if the rerupture rate and the tendon strength are equivalent to operative repair, without the increased risk of infection and wound complications that accompany surgical repair. Acute muscle injuries treated with hematoma aspiration and PRP injection may be another potential indication. A study of this type of injury in elite athletes has been initiated at the authors' institution.

SUMMARY

In summary, PRP has emerged as a promising, but not proven, treatment option for tendon and muscle injuries and disorders. Basic science and animal investigation have begun to help in understanding the mechanism by which PRP affects tissue restoration. Because PRP is autologous and is prepared at the point of care, it also has an excellent safety profile. It may have the ability to transform the care of muscle and tendon injuries in both elite and recreational athletes. Well-designed prospective randomized trials will be required to best understand how, when, and where to use PRP most effectively.

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