Treatment of Tendinopathies with Platelet-rich Plasma

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KEYWORDS

• Tendinopathy • Platelet-rich plasma (PRP) • Biologics • Tendons

KEY POINTS

- Pain and dysfunction related to tendinopathy are often refractory to traditionally available treatments and offer a unique challenge to physicians as there is no current gold standard treatment.
- Injectable biologics including platelet-rich plasma (PRP) may represent a new modality in conjunction with a multifaceted treatment approach.
- PRP injections are not associated with the systemic or tendon degradation risks of corticosteroids or the inherent risks of surgery.
- Basic science studies are promising but have not been replicated with high-powered evidence at the clinical level.
- Given this promise and the lack of a definitive treatment, further evidence to expand understanding of the role of PRP in the treatment of tendinopathy is needed.

CAUSES OF TENDINOPATHY

Tendons serve as the interface between muscles and the skeletal structures on which energy is transferred, ultimately leading to motion. They typically function at 30% to 40% of their maximum tensile strength and are injured when exposed to supramaximal loading.[1] A low metabolic rate allows tendons to function under prolonged stress but can also lead to delayed healing when injury occurs.[2,3] Common terms used in the past to describe tendon disorders include tendinitis, tendinopathy, and tendinosis. Tendinitis, which implies an inflammatory cause, has largely been abandoned as a term because of multiple studies showing a degenerative rather than inflammatory milieu in affected tendons.[4–6] Tendinopathy is an often-used term that implies a painful subacute functional loss that may or may not involve an inflammatory component. Tendinosis refers to the degenerative structural changes that occur in tendons that

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fail to heal after injury. Once thought to represent a persistent inflammatory process, tendinosis is now understood to be characterized by a scarcity of clinical or histologic signs of inflammation and instead shows fibrotic replacement of collagen, poor organization of remaining collagen fibers, increased neovascularity, and a lack of inflammatory mediators.[7–12]

Although the end result is more clear, the pathophysiology of tendinosis is still being debated, because multiple factors likely contribute to the hallmark pain and dysfunction of incomplete tendon healing.[11,12] Structures that are commonly involved include the rotator cuff tendons of the shoulder, the wrist flexor and extensor tendons, and the patellar and Achilles tendons of the knee and ankle, respectively. Like many other types of musculoskeletal disorder, both intrinsic and extrinsic risk factors are associated with the tendinopathy. Common intrinsic factors identified include altered biomechanics, decreased strength, both hypoflexibility and hyperflexibility, increasing age, female gender, certain medication use, and diabetes.[5,13–16] Extrinsic factors including load amount, duration, frequency, and direction of movement also contribute to the risk of developing tendinopathy.[17,18] In addition to pain and dysfunction, studies have shown a profound increase in risk for tendon rupture in degenerated tendons, highlighting the importance of developing effective treatment strategies not only for pain relief but also for tendon healing.[19]

HISTORY OF TENDINOPATHY MANAGEMENT

The classic tendinopathy treatment algorithm focused initially on palliation via arrest of a presumed inflammatory process. Nonsteroidal antiinflammatory drugs (NSAIDs), relative immobilization, topical modalities, compression, and corticosteroid injections are all common interventions used to quell pain and allow participation in a rehabilitation program focusing on stretching and eccentric strengthening. Although pain relief is an important goal in treating the patient with tendinopathy, modalities that are intended to decrease pain in the short term do not address the underlying tissue disorder. The inflammatory cascade that was previously targeted as a means of treating tendinopathy is now understood to be integral in the wound healing process, and blunting its effects may lead to delayed healing and slower functional recovery.[20–23]

It has been theorized that resetting the healing process via the introduction of concentrated growth factors, proteins, and many other bioactive substances, all of which are plentiful in platelet-rich plasma (PRP), may be an effective means of treating recalcitrant tendinopathies. PRP preparations have been used with proven efficacy for years to augment tissue healing in surgical wound closure as well as fat, skin, and bone grafting, and more recently have been used with success in treating tendinopathy as well.[24–28]

What is PRP?

PRP has been defined as an autologous concentration of platelets obtained after gentle centrifugation of whole blood.[29,30] The resultant supernatant contains a high concentration of platelets and the 7 fundamental protein growth factors secreted by the alpha granules of platelets to promote wound healing, including platelet-derived growth factors (PDGF) alpha, beta, and alpha/beta, transforming growth factors (TGF) beta 1 and beta 2, vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF).[31,32] A summary of the functions of these and other bioactive components of PRP is presented in **Table 1**.

The plasma component of the centrifuged supernatant also contains 3 important proteins for tissue regeneration: fibrin, fibronectin, and vitronectin. Fibrin polymerizes

Table 1
Bioactive components of PRP

Factor	Origin	Function
PDGF αα	Alpha granule of platelets	Cell differentiation, neovascularization[33,34]
PDGF αβ	Alpha granule of platelets	Cell differentiation, fibroblast migration, ECM synthesis[34,35]
PDGF ββ	Alpha granule of platelets	Cell proliferation and differentiation, collagen remodeling[34,36–38]
TGF β1	Alpha granule of platelets	Stimulation of collagen formation[34,39]
TGF β2	Alpha granule of platelets	Tendon differentiation[34,40]
VEGF	Alpha granule of platelets	Neovascularization, prevention of apoptosis[34,41,42]
EGF	Alpha granule of platelets	Fibroblast proliferation[41]
Stromal-derived factor 1α	Alpha granule of platelets	Promotes catabolism of degenerative tissue; recruitment of mesenchymal stem cells and fibroblasts[43,44]
Fibrin	Plasma	Component of ECM; stimulation of phagocytosis[47]
Fibronectin	Plasma	Component of ECM; stimulation of phagocytosis[47]
Vitronectin	Plasma	Coordination of cell migration[40]
Interleukin-1β	Macrophage	Increases leukocyte maturation and FGF activity[43]
FGF	Alpha granule of platelets	Neovascularization, stimulation of ECM production and cell migration[34,37,45,46]
IGF-1	Alpha granule of platelets	ECM synthesis, fibroblast proliferation[34,47]
IGF-2	Alpha granule of platelets	ECM synthesis, protein cell proliferation[47]

Abbreviations: ECM, extracellular matrix; FGF, fibroblast growth factor; IGF, insulinlike growth factor.

and cross-links with fibronectin to form the proper substratum for the development of new tissue, and both are also involved in signal transduction to stimulate phagocytosis of designated tissue.[47] Vitronectin coordinates cell migration via proteolysis and cell adhesion[48]

PRP Preparations

Multiple variables can influence the efficacy of PRP, including platelet and leukocyte concentrations, the use of activators, and pH of the injectant. Attempts to determine the optimum platelet product for soft tissue healing are discussed later.

Platelet concentration

Normal platelet concentrations range from 150,000 to 300,000/μL in the healthy adult. Early studies promoted an optimal platelet concentration of 2.5 to 3 times the baseline platelet count and suggested that concentrations higher than this might have an inhibitory effect on healing.[49,50] However, recent research has challenged the use of lower platelet concentrations, suggesting instead the use of preparations of 1.5 million/μL, or 5 to 7 times the typical baseline concentration. Higher platelet concentrations o induce greater angiogenesis and are not inhibitory up to 2 million to 3 million per

microliter, or 10 times the baseline platelet count.[51,52] In addition, Haynesworth and colleagues[53] showed an exponential increase in mesenchymal stem cell proliferation as platelet concentration increased from 2.5 to 10 times that contained in whole blood. The current body of literature suggests greater tissue healing with the use of higher platelet concentrations, although additional well-designed studies are needed to further answer this question.

Leukocyte concentrations

The use of leukocytes in PRP preparations is a topic of continuing debate. There are commercially available platelet-concentrating systems that remove leukocytes from the preparation, which is then termed leukocyte poor. However, other units centrifuge whole blood to yield a leukocyte-rich supernatant that has been shown to contain primarily monocytes and leukocytes, and few neutrophils. The presence of leukocytes has been associated with prolonged soft tissue healing. Enzymatic activation of matrix metalloproteinases (MMPs) by granules released from neutrophils present in leukocyte-rich PRP has been shown to injure soft tissues and delay healing in vitro.[54–56] In contrast, monocytes and lymphocytes have been shown to recruit and promote the differentiation of stem cells in vitro, and it has been theorized that their presence may result in higher stem cell counts and promotion of the wound healing cascade in vivo.[52,57,58] Furthermore, El-Sharkawy and colleagues[59] compared the stimulation of cytokine release (a marker of inflammation) in tissues treated with whole blood, leukocyte-rich PRP, and platelet-poor plasma and exposure to PRP with leukocytes was associated with a decrease in cytokine release. Again, there are not enough well-designed in vivo clinical studies to determine optimum amount and distribution of leukocytes in PRP preparations.

Use of activators

Some clinicians have theorized that activating platelets via the use of thrombin or other activating agents may lead to improved concentrations of growth factors at the site of injection because of concerns for migration of the PRP away from the intended region.[60] Scherer and colleagues[61] examined the effects of platelet activation on wound healing in vivo and in vitro and found that wounds treated with nonactivated PRP healed more quickly, and other studies have yielded similar results.[62] One theory for this finding is the concept of a healing cascade schedule, whereby growth factors may have an optimal effect when present on demand rather than being released immediately at the time of injection. The cells effecting change at the site of healing require different growth factors at different times because their concentrations follow a temporal schedule during the wound repair cascade, as shown in (**Fig. 1**).

pH of injectant

Regarding pH, commercially available PRP preparation systems typically use citrate dextrose for its anticoagulant properties. By binding to free calcium in the blood it arrests the coagulation cascade, stabilizing the blood in a liquid state to allow centrifugation. However, the presence of citrate dextrose in the PRP preparation creates a slightly more acidic injectate, which initially raised concern for impairment of tissue regeneration. The wound healing process is known to involve an acidic environment initially followed by a transition to a neutral and then slightly alkaline pH later.[63,64] The timing of pH variations stimulates the release of growth factors at the appropriate time, as shown by Wahlström and colleagues,[65] who exposed platelets to media of differing acidity and found that, at the lowest pH, PDGFs were more prevalent. PDGFs are important in all stages of wound healing, but are particularly active early.[66] Given the acidic milieu of the targeted tissue, buffering PRP to counteract the acidity of

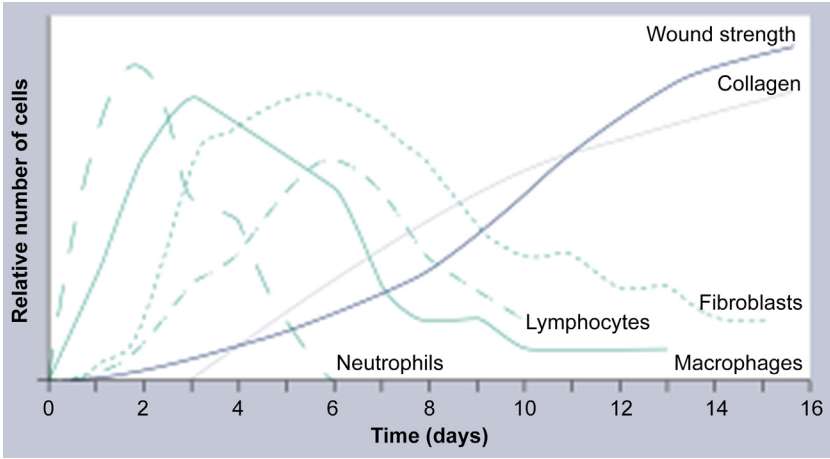

Fig. 1. Leukocytes and Wound Healing. (*From* Mautner A, Malanga G, Colberg R. Optimization of ingredients, procedures, and rehabilitation for platelet-rich plasma injections for chronic tendinopathy. Pain Manag 2011;1(6):527; with permission.)

citrate dextrose is generally viewed as unnecessary, and may even delay soft tissue healing.

Local anesthetics

Given the volume of fluid injected and the sensitive soft tissues targeted with PRP injections, many practitioners use local anesthetic for the procedure. Previous studies have yielded conflicting results on the effects of local anesthetics on wound healing, tenocytes, and platelets. Kevy and colleagues[52] showed noninferiority of platelets when mixed with local anesthetics, but multiple studies have shown decreased tenocyte cell proliferation and decreased cell viability.[67,68] Studies have also associated the concomitant use of methylprednisolone, a corticosteroid sometimes used for relief of the expected postprocedure pain, with decreased tenocyte proliferation and cell viability.[67,69] The authors prefer to use only minimal, diluted anesthetics and no corticosteroids with PRP injections.

Basic Science Studies on PRP

Injectable biologics represent a promising, novel means of treating tendinopathy, a common diagnosis with few overwhelmingly efficacious treatments to date. Although PRP is a proven treatment of surgical wound closure and graft incorporation, as stated earlier, its use has increased in musculoskeletal medicine despite a paucity of prospective, large-scale, randomized controlled clinical trials of high methodologic quality to support its use. However, numerous preliminary proof of concept studies have shown a beneficial effect of PRP on tenocyte healing, and several of these are discussed here.

Many basic science studies have examined the association between treatment with PRP and an increase in mediators that are known to contribute to tissue healing and regeneration. Baksh and colleagues[70] conducted a review of the available literature evaluating the effects of PRP at the cellular level and described multiple studies showing initial increased angiogenesis, improved tenocyte formation, and tendon healing. PRP has been shown to have a positive effect on the expression of several growth factors, including the various PDGFs, TGFs, insulinlike growth factors, hepatocyte growth factor, and VEGF.[31,32,34,71–76]

In theory, the upregulation of growth factors leads to increased formation of tenocytes and the structures that support them, and the current body of literature supports this. Zhang and Wang[77] exposed tendon stem cells to PRP in vitro and found an increased rate of differentiation into tenocytes, increased tenocyte-related gene and protein expression, as well as the production of collagen, and others have shown similar increases in collagen formation.[76–80] Increased vascularity at the site of PRP injection indirectly promotes tendon healing via recruitment of additional platelets, leukocytes, and their associated growth factors, and multiple studies have shown increased angiogenesis in vivo compared with controls with the use of PRP.[81–84] Kajikawa and colleagues[85] showed that this increase was functional, with improved recruitment of native circulation–derived cells with a resultant increase in type I and III collagen levels at the site of PRP application. This finding was confirmed by Bosch and colleagues,[84] who showed not only quantitative changes in collagen but qualitative improvements in the collagen network and increased metabolic activity as well. The improved collagen matrix size and quality has been shown to improve the tensile strength of tendons treated with PRP.[79] In this way the effects of PRP probably extend beyond the mediators in the injectate alone, and this lends support to the longer-term expected recovery compared with the brief time in which PRP is present at the site of injection.

Human Clinical Studies on PRP

For PRP to be clinically relevant, the promising results seen in basic science studies need to translate into improved patient outcomes. Although there is a dearth of well-designed clinical studies of significant power this body of research is expanding understanding of the effects of PRP on tendon healing. Taylor and colleagues[86] reviewed the available literature regarding outcomes following PRP treatment of tendon injury. Thirteen studies met criteria, including 3 randomized controlled trials. Of those, nearly all showed benefit with PRP except for the treatment of chronic Achilles tendinopathy, which yielded conflicting results.

Lateral epicondyle extensor tendinopathy

PRP use in the treatment of chronic lateral elbow pain has been associated with long-term efficacy in multiple studies examining both pain and function,[87–90] including a recent well-powered multicenter double-blind randomized controlled trial by Mishra and colleagues[90] showing statistically significant improvement in pain at 6 months after the procedure compared with percutaneous needling of the tendon. Peerbooms and colleagues[87] also showed in a double-blind, randomized control trial at 2 years' follow-up that individuals receiving a single injection of PRP fared significantly better than individuals receiving a corticosteroid injection for chronic lateral epicondylitis.[91] Ultrasonographic examinations of extensor tendons treated with PRP have also shown promising results for tendon healing, suggesting that preclinical study findings may translate into clinical benefit. A pilot study by Chaudhury and colleagues[92] found morphologic improvements, defined as an increase in vascularity, at the myotendinous junction in all 5 patients evaluated at 6 months after PRP injection (1 subject was lost to follow-up). In contrast, other studies have failed to replicate these results, including a randomized, double-blind, placebo-controlled trial by Krogh and colleagues.[93,94] This study examined the association of either PRP, glucocorticoid, or saline (placebo) injections with pain relief, vascularity, and tendon thickness at 1 and 3 months. Injection of glucocorticoid was associated with superior pain relief at 1 but not 3 months, and decreased vascularity and tendon diameter at 3 months. Neither glucocorticoid nor PRP was superior to saline injection by any measured variable at 3 months. The merits of saline as a true placebo have not been established,

and longer-term follow-up may have revealed different outcomes because the full benefits of PRP cannot be measured at 3 months after injection.[87–92]

Patellar tendinopathy

Chronic patellar tendinopathy causes great pain and functional limitations, and conservative means of treatment including eccentric training have only been estimated to be 50% to 70% effective at restoring pain and function to allow participation in sports.[95,96] As such, it is a potential target for PRP therapy. Clinical studies have shown significant pain reduction, even in patients who had failed other treatments. Gosens and colleagues[97] evaluated improvements in pain prospectively and found significant improvements both in patients who had never sought interventional treatment as well as in those that had been treated surgically or received prolotherapy or corticosteroid injections. Other studies found similar results with augmented pain relief and improved tendinous echotexture under ultrasonography when PRP and physical therapy were both used and injections were performed with ultrasonography guidance.[98–100] Medium-term follow-up found statistically significant improvements in pain and return to sports activity up to 4 years following injection.[101] Unilateral pain and shorter duration of symptoms predicted a positive response, whereas BMI and previous surgical treatment did not significantly affect outcome, all of which are important considerations for appropriate patient selection. A multicenter retrospective review of outcomes after PRP in multiple tendons by Mautner and colleagues[102] showed the least improvement in pain, function, and patient satisfaction with patellar tendon injections (59% vs 81%–100% at other sites).

Achilles tendinopathy

Achilles tendinopathy is a common target of PRP therapy, although its efficacy is a source of continuing debate. Several small studies have shown postinjection improvements in pain, tendon structure, return to activities, and quality of life.[100,102–107] In addition to decreased pain, Ferrero and colleagues[100] found statistically significant structural changes in Achilles tendons 6 months after PRP combined with dry needling treatment including decreased tendon size, decreased echogenicity, and increased power Doppler signal indicating an induced vascular response. Other studies have supported these results, with subjective and objective positive benefits as much as 18 months after PRP.[106] Deans and colleagues[107] used a comprehensive program including PRP followed by 6 weeks of immobilization with therapeutic ultrasonography before beginning an eccentric exercise program. This protocol led to excellent pain relief, improved quality of life, and return to sport at 6 months after the procedure and represents a multimodal treatment approach that may be more effective at relieving chronic Achilles tendinopathic pain and restoring function than PRP treatment alone.

There have been studies that have failed to replicate these positive results and that call into question the role of PRP in treating chronic midportion Achilles tendinopathy. De Jonge and colleagues[108] designed a double-blind, randomized controlled trial evaluating the effect of PRP injection versus a saline injection (deemed a placebo) combined with an eccentric strengthening program on pain, tendon size, and neovascularity. Both groups improved significantly regarding pain and sonographic outcomes at 12 months without a significant difference between treatment groups.[109,110] A review of the literature conducted by Sadoghi and colleagues[111] found no well-designed in vivo evidence to support the use of platelet therapy in chronic Achilles tendinopathy among the 4 studies meeting criteria. A subsequent review by Kaux and Crielaard[112] highlighted the conflicting results found in several studies examining the clinical effects of PRP injection to the Achilles and other tendon targets, as well as the challenges of controlling

variables such as preinjection and postinjection rehabilitation, timing of follow-up, injectate quality, and design of a proper control. Overall, these mixed results highlight the complex nature of tendon healing and the need for further research.

Rehabilitation Following PRP

The use of PRP in the treatment of musculotendinous injuries involves a postprocedure rehabilitation plan that is specific to the treated structure. In general, the affected area is treated as though a new tissue injury has occurred and rehabilitation follows a stepwise progression that follows the stages of wound healing as shown in **Table 2**.[102,108,113,114]

Table 2
After PRP rehabilitation

Phase	Length of Time	Restrictions	Rehabilitation
Phase I: tissue protection	Days 0–3	Consider NWB or protected WB for lower extremity procedures, especially if in pain No weight training Avoid NSAIDS Limited ice	Relative rest Activities as tolerated; avoiding excess loading or stress to treated area Gentle AROM
Phase II: early tissue healing; facilitation of collagen deposition	Days 4–14	Progress to FWB without protective device Avoid NSAIDs Limited ice	Light activities to provide motion to tendon; aerobic exercise that avoids loading of the treated tendon Gentle prolonged stretching Begin treatment on kinetic chain/adjacent regions
	Weeks 2–6	Avoid eccentric exercises Limited NSAIDs Limited ice	Progress weight-bearing activities Low-weight, high-repetition isometrics (pain scale <3/10) Open kinetic chain activities Soft tissue work to tendon with CFM, IASTM Dynamic stretching
Phase III: collagen strengthening	Weeks 6–12	—	Eccentric exercises (keep pain scale <3/10) 2 sets of 15 repetitions 2/d then increase to 3/d Closed kinetic chain activities Plyometrics; proprioceptive training and other sport-specific exercises Progress weight-bearing activities and consider return to sport if pain <3/10
	Months 3+	Reassess improvement; if not >75% improved consider repeat injection and return to phase I	Progress back to functional sport-specific activities with increasing load on tendon as pain allows

Abbreviations: AROM, active range of motion; CFM, cross-friction massage; FWB, full weight bearing; IASTM, instrument-assisted soft tissue massage; NWB, non–weight bearing; WB, weight bearing.

In the immediate postinjection period the inflammatory cascade is activated. Mediators from the injected PRP as well as the body's inherent immunologic response result in a milieu rich in cytokines and growth factors necessary for tissue healing. This phase typically lasts up to 72 hours, during which time patients are advised to use acetaminophen and other oral agents that do not affect the inflammatory cascade for subjective pain relief. There is a theoretic risk of disruption of the fibrin plug that develops during the initial healing response, and, as a result, a degree of immobility of the affected tissue is typically recommended for the first few days, although there is little evidence to refute or support this practice.

Over the following six weeks, the secondary immune response consisting of the sustained recruitment of lymphocytes, polymorphic neutrophils, and macrophages results in proteolytic degradation of injured tissue. Fibroblasts also begin to secrete precursors of the extracellular matrix during this, the proliferative phase. Initial rehabilitation during this phase prepares the tissue for remodeling. After the period of immobilization described earlier, controlled passive range of motion exercises are recommended and evidence exists to suggest that motion and mild stretching guide nascent tendinogenesis and remodeling.[115] At two weeks after injection the patient can begin to undergo soft tissue massage to the affected tendon and can begin low-intensity strengthening exercises, both under the guidance of a physical therapist.

The third and final postinjection phase involves the synthesis, accumulation, and organization of the type I collagen fibers that will make up the nascent healthy tendon.[116] This maturation phase is expected to last several months and the patient should be counseled to allow adequate time for tissue healing. The timing of initiating eccentric strengthening exercise has been debated and positive outcomes have been documented after as early as one week and as late as six weeks.[87,89,117–119] There is some evidence that eccentric loading may subject the injured tendon to excessive force and hinder early angiogenesis. In theory, if performed too early, this could negatively affect the healing cascade.[118] The authors recommend waiting until six weeks after the procedure to initiate eccentric exercise. Return to preinjection athletic pursuits should follow symptomatic improvement, because linear improvements in pain and function are expected during the first six months after the procedure. Over time, continued tendon healing and symptomatic improvement are expected with evidence to support minimal risk of rupture.[91]

Patients typically require no more than one PRP injection in the setting of adequate rest followed by gradually progressive rehabilitation. However, in certain cases, especially in patients who experienced a partial response to the first injection, a second or even a third injection can be considered for continued improvement. Postprocedure evaluation at six to eight weeks commonly finds the patient with only partial benefit, and there is no clear evidence-based protocol regarding reinjection. It has been the author's experience that subjective improvement of greater than 50% at eight weeks and 75% at twelve weeks has been associated with long-term pain relief and functional improvement without the need for additional injections. Further objective sonographic tendon improvements often cannot be visualized at the time of initial follow-up and are generally not used to determine the need for additional injections.[100,103,106] Complete tendon remodeling may take up to two years to occur, leaving the clinician with few objective data to guide the decision to repeat the procedure, which highlights the need for well-designed and well-powered studies to establish injection criteria that leave both physician and patient confident in the expected outcome.

SUMMARY

Pain and dysfunction related to tendinopathy are often refractory to traditionally available treatments and offer a unique challenge to physicians, because no current gold standard treatment exists. Injectable biologics, including PRP, may represent a new modality in conjunction with a multifaceted treatment approach. PRP injections are not associated with the systemic or tendon degradation risks of corticosteroids or the inherent risks of surgery. Basic science studies are promising but have not been replicated with high-powered evidence at the clinical level. Given this promise and the lack of a definitive treatment, further evidence to expand understanding of the role of PRP in the treatment of tendinopathy is needed.

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