Role of Mechanical Loading for Platelet-Rich Plasma-Treated Achilles Tendinopathy

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Abstract

There is no consensus on the optimal rehabilitation protocol after platelet-rich plasma (PRP) treatment for tendinopathy despite basic science studies showing the critical role of mechanical loading in the restoration of tendon structure and function posttreatment. In this article, we will review tendon mechanobiology, platelet biology, and review levels I and II Achilles tendon clinical studies paying particular attention to the role of mechanical loading in rehabilitation of injured tendons. Animal studies emphasize the synergistic effect of mechanical tendon loading and PRP to treat tendon injury while clinical studies described minimal details on loading protocols.

Introduction

Tendinopathies are common debilitating tendon conditions, in both the athletic and aging populations, which reduce a patient's ability to work and participate in sports (1). Treatment options traditionally have been palliative and include nonsteroidal anti-inflammatory drugs (NSAIDs), extracorporeal shockwave therapy (ESWT), corticosteroids, and surgery. Platelet-rich plasma (PRP) injections have emerged as an alternate to these traditional treatments. PRP belongs to a class of interventions called orthobiologics, which use biological substances aimed to foster healing in injured musculoskeletal tissues. Orthobiologics have since expanded to include the use of tissues harvested from bone marrow, adipose tissue, and placenta, among others, but many physicians still regard PRP as the "original" orthobiologic. PRP for tendinopathy involves an injection of concentrated autologous or allogenic platelets (PLTs) to boost one's tendon healing capacity (2–7). While animal investigations generally show the efficacy of PRP treatment for tendinopathy, clinical results have been variable (8–12). Variations in PRP preparation, cellular content, frequency and number of injections, and subtypes of treated tendon injuries may account for the variability in clinical studies. This manuscript will review both the role of mechanical loading and PRP biology for tendon, and will appraise levels I and II clinical studies for PRP-treated Achilles tendinopathy, paying specific attention to the post-PRP rehabilitation program. Achilles tendinopathy was chosen as this tendon is subject to heavy mechanical loading, and a large portion of Achilles tendon injuries fail conservative management and become chronic (13).

To find level I and II studies on PRP for Achilles tendinopathy, a search was conducted on PubMed on December 10, 2018, using the terms: tendon OR tendinopathy OR tendinosis OR tendinitis AND platelet-rich plasma OR PRP OR autologous conditioned plasma OR autologous conditioned plasma (ACP). Titles and abstracts were screened, and the following inclusion criteria were used: human studies, randomized controlled trials, and prospective or retrospective cohort studies using PRP or ACP products for the treatment of Achilles tendinopathy using ultrasound guidance. Studies using PRP combined with surgery and those that did not use ultrasound guidance were excluded. The 88 full texts were read and relevant data on mechanical loading rehabilitation was extracted. This yielded five publications for the review. There were 35 level I and II studies pertaining to other tendons (20 on common wrist extensor tendon, 8 on patellar tendon, 3 on rotator cuff tendon, 2 each on gluteal and proximal hamstring tendons).

Discussion

Tendon Mechanobiology

Healthy tendon tissue displays parallel collagen fibers (65% to 80% of the dry mass is type 1 collagen) among cellular components, including mature tendon cells or tenocytes and tendon-specific stem/progenitor cells (TSCs), within a well-organized extracellular matrix (ECM) composed of proteoglycans, glycoproteins, and elastin (14–19). The highly organized structural components and cellular organization are vital for

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the optimal function of tendon to act as load-bearing units. The mechanical signals are transferred to tendon cells, which, in turn, are transduced to intracellular biochemical responses known as mechanotransduction (20–22). Tendon cells play a vital role in mechanotransduction and maintain tendon tissue homeostasis. Although tendon structure is optimized to support tensile load, excessive loading, defined as a mismatch between load capacity and load placed on the tendon, results in overuse tendinopathy which presents as varying degrees of pain and loss of functional capacity. The tendon cells sense changes in the load and biochemical factors, resulting in a cascade of cellular and matrix responses that initiate pathological consequences (23–27). The mechanisms of tendon injury and repair have been reviewed extensively (5,28–33). According to the pathoetiology of the inflammatory model of tendinopathy, when injured, tendon healing typically progresses through three stages. The first stage is the inflammatory phase which is characterized by acute inflammation as proinflammatory cytokines attract blood cells to begin repair of injured tissue (34). This stage generally lasts for 5 d to 7 d. The second stage is the proliferative phase where the tendon attempts to heal itself via synthesizing reparative, smaller diameter type III collagen and proteoglycans (35). This is believed to take place starting 1 wk after the injury and lasting for approximately 6 wk. The third stage is the remodeling phase where healed tendon undergoes reorganization and crosslinking of mature collagen fibers (36). The remodeling phase is essential for restoring normal tendon mechanical properties. However, healing can fail. When this occurs, the tendon can go into a state of "tendinosis," characterized by a combination of increased mucoid ground substance, collagen disarray, increased type III to type I collagen ratio, neovascularization, and decreased tenocyte density and dysmorphism (19,33,37,38). Cook et al. (29,30) has proposed a continuum model for tendon pathoetiology that explains most clinical presentations. This model has three stages featured as reactive tendinopathy, tendon disrepair, and degenerative tendinopathy. The first stage is the proliferative response in the cell and matrix, the second describes an attempt to heal with matrix breakdown, and the third with a progression of changes in matrix and cells. Regardless of the school of thoughts regarding tendon healing, the failed tendon is characterized by nontenogenic tissue types, such as lipoid, fibrinoid, fibrocartilaginous, or calcified material and is colloquially referred as scarred (35,39). Tendons with nontenogenic tissue have been known to exhibit inferior mechanical properties, such as tensile strength, and they can become chronically painful (40,41) (Table 1).

Tendon is a biological structure that transmits mechanical loads for joint movements. Controlled mechanical loading on tendon, typically accomplished with physical therapy (PT) for clinical patients, benefits tendon healing, presumably through its effect on both TSCs and ECM. The effect of mechanical loading on TSCs is magnitude-dependent. In an *in vitro* mechanical loading model, it has been shown that small mechanical stretching (~ 4%) induces TSCs to differentiate into tenocytes, whereas large mechanical stretching (~8%) results in differentiation of TSCs into nontenocytes; these findings may explain the development of tendinosis (39). In rats, the benefit of mechanical loading on tendon appears to take place as early as 4 wk after initiation of the tendon loading program (42). Specific mechanisms involve decreased nontenogenic transformation and increased TSC activities evidenced by downregulation of nontenocyte genes (collagen II, PPARγ, SOX-9, and Runx-2) and by upregulation of tenocyte-related genes (collagen I and III) (43). Mechanical tendon loading also benefits the ECM via activation of fibroblasts that increase the secretion of transforming growth factor-β1 (TGF-β_1), connective tissue growth factor (CTGF), and insulin-like growth factor-I (39,42,44). These growth factors ultimately aid collagen cross-linking and help to restore preinjury tendon tensile strength (42). Hence, placing tendons under moderate mechanical loading is beneficial for tendon healing. It is important to keep in mind that as compared with young adult tendons, aging tendons may not respond similarly to mechanical loading because they have inherently reduced mechanical strength (36) and a decline in the number and quality of TSCs (45), which likely make aging tendons more susceptible to impaired or slower healing and tendon homeostasis that favors development of tendinosis (38,46).

Effect of PLTs on Tendon Remodeling

PLTs are formed from megakaryocytes through hematopoiesis and are synthesized in bone marrow by pinching off from their progenitor cell (47). Thereafter, they are released in a nonthrombogenic state into the peripheral circulation as anucleate, small discoid blood cells. The average PLT count in adults ranges from 150 to 350 $\times$ 10^6/mL of circulating blood and PLTs have been recognized for their pivotal roles in the healing cascade (48). PLTs have a number of intracellular structures, including alpha-granules, comprised of PLT-derived growth factors (PDGFs) and angiogenesis regulators, and dense granules containing adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, histamine, calcium, and mitochondria. Other complex PLT biological components include adhesins and coagulants as well as immunological molecules (47). These molecules serve a multitude of functions, first within the clotting cascade and finally as initiators of tissue healing processes. PLTs are able to detect injuries via endothelial-driven cellular reactions and are able to access bodily tissues, including tendon via vascular flow. Following an injury, PLTs are activated, releasing the alpha granular contents, including PDGF, TGF-, vascular endothelial growth factor (VEGF), epidermal growth factor-1, fibroblastic growth factor (FGF), CTGF, and hepatocyte growth factor (39,49). The biological activities and the individual specific functions of the various PGFs have been described extensively (47–51).

PLTs also contribute to many adjunctive and supportive activities that result in increased angiogenesis and vascular remodeling through release of several chemokines and cytokines, via paracrine, autocrine, and endocrine modes of action (51,52). Because of these unique modes of action, PDGFs also are capable of exerting morphometric and mitogenic effects on multiple cell types, and they play important roles in tendon repair (47,49). Chemokines and cytokines also play vital roles in tendon regeneration and tendon pain modulation although the discussion of these is beyond the scope of this article (50).

PRP in Animal Tendons

While a clear definition is not currently available, PRP is most simply defined as "a preparation of a small volume of plasma with an increased concentration of PLT from

Table 1.
Tendon healing cascade, which is reinitiated after PRP injection, and the goals and methods implemented during rehabilitation to optimize tendon healing.

Phase of Tendon Healing	Timeline (Postinjury)	Pathophysiology	Rehabilitation Goals	Rehabilitation Methods
Inflammatory	Days 1–7	Migration of erythrocytes and inflammatory cells (monocytes and macrophages predominate. Necrotic debris removed. Tenocytes migrate to the wound.	Pain control Tissue protection	Absolute or relative rest Cryotherapy[a] NSAIDS[a]
Proliferative	Weeks 1–4	Proteolytic degradation. Increased neovascularization. Stimulation of fibroblasts synthesizing type III collagen.	Mechanical stimulation in controlled tendon loading Neuromuscular reeducation	Stretching Strengthening (isometric, eccentric, concentric) Proprioceptive training
Remodeling	Begins at ~4–6 wk.	Functional tissue laid down (type I collagen replaces type III collagen).	Return to normal activity	Sport-specific training
Unsuccessful healing	Can begin at any point	Normal tendon tissues are replaced with nontenogenic tissues including lipoid, fibrinoid, fibrocartilaginous, or calcified material resulting in scar.	Avoid this phase	Difficult to treat

[a] Often discouraged after a regenerative medicine procedure to allow the inflammatory cascade to occur.

autologous or allogenic hosts" (53). In animal studies, PRP infiltration in acutely injured tendons promoted shortening of the inflammatory phase, with additional benefits during the proliferative phase, such as collagen maturation noted by increased type I to type III collagen ratio and increased ECM synthesis, resulting in faster healing (7,10,43). PRP's ability to promote angiogenesis has been studied extensively showing that PRP infiltration results in increased vessel density as early as 2 wk after the intervention (54). Since reduced vascularity of tendons is a major factor in their limited healing capacity, PRP-associated angiogenesis also contributes to accelerated tendon healing (54).

Optimum PRP Formulation: PLT Concentration, White Cell Inclusion, and Timing of Injection

Optimal PLT concentration is a frequently discussed topic among practicing clinicians. This likely depends on both 1) the biology specific to the target tendon and 2) tendon injury type, in terms of severity and chronicity. One recent study out of Japan revealed that 1.0×10^6/μL PLT concentration was more effective in both pain control and tendon regeneration in rat patellar tendons than 5.0×10^5/μL, supporting the notion that higher PLT concentration is more effective (55). On the other hand, an extremely high concentration of PLT has been shown to result in detrimental effects on tendon proliferation; therefore, continued investigations are necessary to elucidate the optimum PLT concentration (56).

Leukocytes have a great impact on the intrinsic biology of tendons because of their immune and host-defense mechanisms. The presence of various leukocytes in PRP can have a significant effect on tendon healing. In PRP, lymphocytes, which produce insulin-like growth factors that support tissue remodeling (57) are more concentrated than other leukocytes.

Monocytes are noninflammatory leukocytic cells and are the precursors to macrophages, which are important cells of the immune system, similar to neutrophils. A distinct difference between the cells is that monocytes do not lead to a prolonged inflammatory condition but instead play important roles in tissue healing. M1 macrophages are responsible for producing several inflammatory cytokines that support host defense through pathogen clearance, necrotic tissue clearance, and reactive oxygen species (58). Additionally, the M1 macrophage phenotype produces VEGF and FGF (58). M2 macrophages have anti-inflammatory capacities and generate precursors for collagen and fibroblast-stimulating factor, thus supporting their role in ECM deposition (58). Monocytes/macrophages release additional proregenerative growth factors that lead to neovascularization, proliferation of myogenic precursor cells, and play key roles in wound repair and inflammatory control (59). Therefore, the presence of high concentrations of monocytes/macrophages in PRP is likely to contribute to better tendon healing.

Neutrophils play a key role in various healing cascades by forming a dense barrier against invading pathogens and counteracting infections (60). Their presence in PRP can be desirable within specific treatment protocols that require higher levels and longer periods of inflammation such as fracture healing (61), while it can be harmful and not indicated in other applications. In fact, animal studies demonstrated that the use of neutrophil-rich PRP resulted in a higher collagen type III/collagen type I ratio, leading to fibrosis and decreased tendon strength (62).

Another frequently discussed topic among clinicians is the timing of PRP application and the exact clinical indications. Two studies by Zhang et al. (63,64) are insightful in this regard. For example, after acute tendon injury, PRP induced tenogenic differentiation of TSCs and suppressed nontenocyte differentiation. However, when PRP was applied to TSCs from tendons that had already undergone nontenogenic differentiation, PRP was unable to reverse the undesirable differentiation that had already occurred. These findings imply that PRP may not be effective in repairing tissues once injury has progressed to the chronic stage, and it may not be indicated in treating tendinosis (63,64).

The differences in PRP composition and quality among numerous preparation methods remain unclear. Specifically, the benefit of including leukocytes in the PRP product remains controversial, and few studies have evaluated the effects of the interaction between PLTs and leukocytes on the growth factor concentrations, proinflammatory effects, and cellular effects.

Several studies, mainly in the orthopedic field and sports-related injuries, support the use of leukocyte-poor (LP) PRP, whereas leukocyte-rich (LR) PRP had a leading role in biological processes associated with healing, including angiogenesis and matrix remodeling. Kobayashi et al. (65) concluded that the leukocyte concentration positively correlated with PDGF-BB and the VEGF concentration, while it negatively correlated with FGF-b.

In a study by Yan and co-workers (66), it was revealed that LP-PRP in a rabbit chronic tendinopathy model lead to larger collagen fibril diameters than when LR-PRP was used. Whereas in both the LP-PRP and LR-PRP groups significantly lower matrix metalloproteinase (MMP)-1 and MMP-3 expression levels were seen than in the control group. Stronger chemotactic and proliferative properties of PRP seem to be present with LR-PRP, with tendinopathic cells migrating at a higher velocity under LP-PRP conditions, although this formulation is more proinflammatory in terms of IL-6 secretion (67).

Based on different PRP formulation profiles and measurable effects in LP and LR-PRP, emphasis should be placed on the temporal needs and biological characteristics of injured tendons, and PRP formulations should be tailored accordingly, using versatile PRP devices, allowing for the preparation of different PRP formulations.

Data Summary of Combining Mechanical Loading and PRP

The efficacy of PRP is shown to depend on mechanical loading. Injection of PRP alone without mechanical loading was found to improve mechanical properties of rat tendons including stiffness and increased stress at failure by days 3 to 5 (11). However, mechanical loading was required for this effect to continue to 14 d. The unloaded tendons were less than one third as strong as the tendons that were loaded normally; without differences in tendon size, stiffness, force, or stress to failure at 14-d follow-up (11).

Clinical Study Review of PRP Injection for Achilles Tendon

Six studies met the initial search criteria as detailed in the introduction section. The study by Kearney et al. (68) was excluded from our review on the basis of lack of guided injection. Details of the five remaining included studies are found in Table 2 (69–73). Four studies treated a total of 98 cases of mid-portion Achilles tendinopathy while one study by Erroi et al. (72) treated 21 cases of insertional Achilles tendinopathy. PRP cellular profiles were not available in any of these studies despite the recent consensus statement on the minimum reporting requirement for orthobiologic trials (74). Based on the PRP kits used; however, injected PRP was likely LP formulation with PRP concentration ranging between 1.6 and 5 times the physiological PLT concentrations (75). Two earlier studies by de Vos et al. and Krogh et al. used a single PRP injection while studies by Boesen, Erroi, and Abate (69,70,72,73) used multiple (two to four) PRP injections 1 to 2 wk apart. Four studies used tenotomy (3 to 10 passes) in addition to PRP as the intervention. Boesen's study was the only study that used four biweekly peritendinous PRP infiltrations without tenotomy (71). PRP plus tenotomy was found to be superior to tenotomy alone at week 24 (73), while PRP plus tenotomy was equal to normal saline injection (69,70) and two biweekly PRP plus tenotomy treatments were equal to three weekly ESWT treatments at 24 wk (72). As for peritendinous PRP injections, PRP was superior to saline injection at week 6 and week 12 but not at week 24 (71). In the same study, high-volume image-guided injection, where a combination of a local anesthetic and corticosteroid was injected to the space between Kager's fatpad and Achilles tendon was more effective than PRP at weeks 6 and 12, and equally as effective at week 24 (71).

The Trend in Clinical Studies: Mechanical Loading Programs

During the post-PRP phase, duration of relative rest or protected loading varied significantly among studies and ranged from Errois' no rest protocol (72) to 4 d relative rest. All except for one study recommended in the "2 d to 4 d" range (69–71,73). Erroi et al. (72) returned subjects to activity with no rest following an intratendinous Achilles tendon PRP injection. With this protocol, one concern is the risk of tendon rupture due to the mechanical stress from intratendinous injection. In some subjects, tendon loading may not be feasible due to procedure-related pain. The first day of formal rehabilitation fell on days 4 to 8 after the last PRP intervention, and it most commonly involved Alfredson's (76) progressive eccentric tendon loading program at its core, although they were all unsupervised home exercise programs. Of our interest was that 10 studies out of 36 non-Achilles tendinopathy PRP level I to II trials (mainly common extensor tendinopathy) had no mention on the post-PRP rehabilitation program (77–86), highlighting the possibility that researchers might not have fully appreciated the rehabilitation program as an integral part of clinical trial protocol involving regenerative strategy.

Conclusions

Animal studies have pointed out that mechanical loading is regenerative to tendons, and the load is synergistic to PRP injections for tendon healing. While Achilles tendon PRP trials have done well in incorporating mechanical loading/post-PRP rehabilitation as part of a regenerative strategy in the studies, the lack of supervision leaves something to be desired. Supervised rehabilitation programs seem to result in increased exercise compliance (87) and improved outcomes, and also it allows for improved ability to monitor for exercise "dosing" (88–91). Although no evidence exists, pre-PRP mechanical loading also can be considered as another method to optimize the regenerative benefit from PRP injections. Implementation of a structured, formal loading program might promote activation of TSCs and potentially improve the outcomes from the subsequent interventions if patients are able to tolerate the program (39,42,44).

In summary, efficacy of PRP injection for tendon injuries might be potentiated when it is used as a part of the spectrum of care where mechanical loading is combined with PRP.

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Table 2.
Detailed description of prehabilitation and rehabilitation protocols after PRP for included level I and II Achilles tendinopathy studies.

Ad Article, Type of Study (Year)	Tendon	n	Procedure Details	Pre-PRP	Postprocedure Meds	Rest Period	Rehab Initiation	Supervised Program?	Rehab Protocol	Outcome
de Vos et al. (69), RCT (2010)	Achilles	PRP: 27 Saline: 27	1 PRP injection vs 1 saline injection Intratendinous	No prior PPR injections. No prior heavy load eccentric exercise program.	Acetaminophen encouraged	2 d	Day 8	No	1 wk of stretching exercises. Daily eccentric training (180 reps) for 12 wk. Gradual return to sports at 4 wk.	VISA-A significantly improved in both PRP and placebo groups after 24 wk without a significant difference between groups.
Krogh et al. (70), RCT (2016)	Achilles	PRP 12 Saline: 12	1 PRP injection vs 1 saline injection Intratendinous	No prior Achilles tendon surgery	N/A	4 d	Day 5	No	Eccentric strengthening, stretching, and coordination	No statistically significant difference in VISA-A between groups at 3 mo. Large dropout rate after 3 mo.
Boesen et al. (71), RCT (2017)	Achilles (midportion, avg duration = 27.5 mo)	PRP: 20 HVI: 20 Placebo: 20	4 PRP injections vs 1 HVI and 3 SubQ saline injections vs 4 SubQ saline injections, all 2 wk apart Peritendinous	No steroid or blood product injection within previous 6 mo. No use of quinolones within previous 6 mo.	N/A	3 d	Day 4	No but PT consulted on days 1, 14, 28, and 42 to make adjustments	Twice daily eccentric training (180 reps) for 12 wk. Maintenance eccentric exercises 3 times per week (weeks 12–24).	VISA-A improved in all groups, greatest in HVI at 6 and 12 wk and HVI and PRP at 24 wk VAS improved in all groups, greatest in HVI and PRP groups. Tendon thickness decreased in HVI and PRP groups only. Muscle function (via Heel Rise test) improved in all groups.

Erroi et al. (72) retrospective cohort (2017)	Achilles (insertional, avg duration = 14 mo)	PRP: 21 ESWT: 24	2 PRP injection (2 wk apart) vs 3 sessions of ESWT Intratendinous	No foot surgery or CSI within previous 3 mo. No anticoagulant or anti-PLT medication within previous 3 mo.	Acetaminophen encouraged Avoid NSAIDs	None	N/A	No	5 phase daily eccentric training: 1) Calf stretching, 2) eccentric exercises, 3) foot proprioceptive exercises, 4) calf stretching, 5) icing for 8 wk. Maintenance protocol as above twice weekly (weeks 8–12). Return to sport after 4 wk if minimal or no pain.	VISA-A improved in both groups up to 6-mo follow up. No differences between groups at 2 or 6 mo follow up but ESWT showed better improvement than PRP at 4 mo follow up. VAS improved in both groups without a difference between groups. Patient satisfaction progressively improved in both groups at all time points.
Abate et al. (73), retrospective observational (2018)	Achilles (noninsertional, avg duration = 12.7 mo)	PRP: 46 Dry Needling: 38	3 weekly PRP injections vs dry needling Intratendinous	No PT modalities or eccentric training within previous 3 mo. No steroid or hyaluronic acid injection within previous 3 mo	Acetamino-phen encouraged Avoid NSAIDs	3–4 d	Day 5	No	Daily eccentric training and stretching (3 sets of 15 reps) for at least 3 mo. Gradual return to sport.	No differences in pain and function between groups at 3 and 6-mo follow up. Patient satisfaction was higher in PRP group at 6 mo (41.3% vs 26.3%).

ACP, autologous conditioned plasma; avg, average; CSI, corticosteroid injection; DASH, Disability of the Arm, Shoulder, and Hand score; HVI, high-volume injection; hx, history; MCID, minimal clinically important difference; mHHS, modified Hip Harris Score; N/a, not applicable; Prehab, prehabilitation; Rehab, rehabilitation; SubQ, Subcutaneous; VAS, visual analog scale; VISA-A, Victorian Institute of Sport Assessment-Achilles; VISA-P, Victorian Institute of Sport Assessment-Patellar.

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