Augmenting tendon and ligament repair with platelet-rich plasma (PRP)

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Summary

Tendon and ligament injuries (TLI) commonly occur in athletes and non-athletes alike, and remarkably debilitate patients' athletic and personal abilities. Current clinical treatments, such as reconstruction surgeries, do not adequately heal these injuries and often result in the formation of scar tissue that is prone to re-injury. Platelet-rich plasma (PRP) is a widely used alternative option that is also safe because of its autologous nature. PRP contains a number of growth factors that are responsible for its potential to heal TLIs effectively. In this review, we provide a comprehensive report on PRP. While basic science studies in general indicate the potential of PRP to treat TLIs effectively, a review of existing literature on the clinical use of PRP for the treatment of TLIs indicates a lack of consensus due to varied treatment outcomes. This suggests that current PRP treatment protocols for TLIs may not be optimal, and that not all TLIs may be effectively treated with PRP. Certainly, additional basic science studies are needed to develop optimal treatment protocols and determine those TLI conditions that can be treated effectively.

KEW WORDS: tendon and ligament injury, tendinopathy, PRP, growth factors.

Introduction

Tendon and ligament injuries (TLIs) are among the most common health problems affecting the adult population[1]. About 16.4 million tendon and ligament injuries occur in the United States every year[2] and the incident rate of TLIs is expected to rise drastically as the population ages and remains active.

Once injured, tendons and ligaments do not completely re-gain the biological and biomechanical properties of normal tendons and ligaments due to the formation of scars and adhesions [3,4]. This abnormality in structure/arrangement after healing often makes the tissue susceptible to re-injury[5]. Another issue is the relatively slow rate of tendon/ligament healing after TLIs, which is most likely due to their poor vascularization compared to other connective tissues[6]. These problems associated with the healing of TLIs pose a considerable challenge for clinicians.

In recent years, a promising new treatment option using platelet-rich plasma (PRP) has been widely used in clinics to treat TLIs[7-13]. PRP is the plasma fraction of autologous blood containing high concentration of platelets and growth factors. After activation, PRP delivers various types of growth factors to injury sites, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), fibroblastic growth factor (FGF), and hepatocyte growth factor (HGF)[14-17]. Because most of these factors are involved in the repair of tendon and ligament injuries[15,18] high concentrations of these growth factors are considered to accelerate TLI healing[19,20]. During healing, tendons are responsive to circulation-derived or locally produced growth factors, most of which are found within PRP[17,21-25]. Therefore in recent years PRP application has been used as a strategy to enhance the healing of injured tendons.

Because PRP is prepared from autologous blood, it is inherently safe. Activated PRP acts not only as a native carrier of multiple growth factors, which stimulate cell proliferation, but also as a 3-dimensional bioactive scaffold (fibrin gel) with a mesh-like microstructure, which enhances cell migration and incorporation[26]. Another presumed advantage of PRP is that unlike individually-isolated growth factors that are used in arbitrary concentrations during treatments, multiple growth factors in PRP are present in physiological proportions, with a natural balance of proliferative and inhibitory agents[27]. Additionally, PRP preparation via centrifugation has been greatly simplified in

recent years, thanks to the development of commercial PRP preparation devices. These technological advances have allowed PRP treatments to move from operating rooms to outpatient offices, and PRP can now be produced easily and safely in 15-30 minutes[14]. As a result, PRP therapy is now widely applied in orthopaedics and sport medicine to treat TLIs.

Many *in vitro* and *in vivo* studies on mammalian models have shown that PRP treatments heal TLIs by enhancing collagen gene expression, stimulating angiogenesis, increasing cell migration, differentiation, and proliferation, and increasing extracellular matrix production[15,28,29]. Clinically, only a few studies have shown promising results on the application of PRP to treat TLIs [7,10-12,30-32].

To better understand how PRP may influence the healing of injured tendons and ligaments, we analyzed the existing evidence on the efficacies and mechanisms of PRP's action gained through basic science studies and clinical trials. In this review, the characterization of PRP is presented first. Next, experiments from basic science studies and the mechanisms by which PRP repairs TLIs at the molecular, cellular, and organismal (animal model) levels are discussed. Then we present clinical applications of PRP in tendon and ligament healing including its uses in the treatment of acute and chronic tendon injuries. Finally, based on these studies, we comment on the existing basic and clinical findings, and suggest future directions for basic research and clinical applications of PRP for the treatment of TLIs.

The PRP terminology

PRP has been described in the literature under different names and abbreviations. Some authors define PRP as only platelets, whereas others note that PRP also contains increased concentrations of leukocytes, fibrin, and some bioactive proteins[31]. Based on these descriptions, PRP was classified into pure platelet-rich plasma (P-PRP), leukocyte- and platelet-rich plasma (L-PRP), and leukocyte- and platelet-rich fibrin (L-PRF)[33,34]. Still, some authors regard autology and growth factors as unique advantages of PRP and prefer the terms autologous platelet concentrate (APC), or plasma-rich growth factors (PRGFs)[27,35]. Based on whether PRP is activated to form gel or not, PRP was also divided into platelet plasma (non-activated platelet) and platelet gel or PRP clot (activated platelet)[17]. Various other terms are also used in the literature, including autologous growth factors (AGF)[36], platelet-leukocyte gel (PLG)[37], autologous platelet gel (APG)[38], platelet-rich gel (PRG)[39], and platelet-rich fibrin (PRF)[32]. In view of the general confusion over PRP terminology, it should be emphasized that different preparations of PRP may have the same name despite having different properties, while similar preparations of PRP might have different names despite having the same properties.

Platelet concentration in PRP

By definition, PRP contains a higher concentration of platelets than baseline levels in whole blood and is the most important feature of PRP. Although it would seem intuitive that a higher platelet count would yield more growth factors and better clinical outcomes, this is still under debate. A study on the efficacy of PRP treatments with different platelet concentrations (1.34× *vs* 1.80×) used to repair patellar tendons in rats showed no significant difference between the 1.34× group and the control group treated with whole blood[40]. Only the group treated with higher platelet concentration (1.80×) showed significantly higher ultimate tensile load and energy absorbed to failure. Kajikawa[29] treated rat patellar tendons using PRP with platelet concentration 8.8 times the number of platelets in whole blood ($523.8 \times 10^4/\mu l$ in PRP, and $59.3 \times 10^4/\mu l$ in the whole blood), and showed that even at such high platelets concentrations, PRP could increase cell proliferation and enhance cell migration significantly when compared to the control group.

However, some authors recommend not using an overly-high platelet concentration for tissue healing. A platelet concentration about 4-5 times higher than baseline concentration was sufficient to enhance bone and soft tissue healing[41], while higher concentrations did not further enhance healing[42]. It was reported that a lower concentration of 2.5 times the baseline levels was optimal to induce cell proliferation of osteoblasts and fibroblasts, with higher concentrations (3.5×, and 4.2-5.5×) resulting in reduced numbers of osteoblasts and fibroblasts[43]. More research is therefore needed to determine the optimal biological effects of PRP with different platelet concentrations in different conditions.

The components of PRP

Both the cellular and molecular components of PRP are well described in many reviews. In this review, we present only a concise summary of these components. More detailed information on individual or combined components is provided by Molloy[23], Alsousou[16,44], and Taylor[19], among others.

Cellular components

The main cellular components of PRP are platelets, and white blood cells (leukocytes). Platelets, which are the critical components of PRP, are not only a source of growth factors and chemokines, but also express chemokine receptors that regulate inflammatory response[45].

In addition to platelets, PRP preparations often contain white blood cells that cause biological effects that differ from PRP preparations without white blood cells. To differentiate between the two PRP prepara-

tions, some authors use terms such as leukocyte- and platelet-rich plasma (L-PRP) and pure platelet-rich plasma (P-PRP)[34,46]. Most current commercial PRP systems, including SmartPReP (Harvest, USA)[47], PCCS (3I, USA)[48], GPS (Biomet Biologics, USA)[49], and Curasan (Kleinostheim, Germany), produce L-PRP[48].

The leukocytes in PRP include neutrophils, monocytes, macrophages, and lymphocytes, which contribute to bacterial killing[39,50]. Leukocytes also impart other beneficial effects on tendon healing: when ACL fibroblasts were cultured in the presence of P-PRP and peripheral blood mononuclear cells (PBMC, consisting of monocytes, macrophages, and lymphocytes, but not neutrophils), significant increases in type I and type III procollagen gene expression, collagen protein production, and cell proliferation were observed, in comparison to the group cultured with only P-PRP. This was accompanied by increased expression of IL-6, which is known to stimulate collagen synthesis in tendons[51,52]. It should be noted that the anabolic effects of PBMCs on ACL fibroblasts were observed only in the presence of platelets, and such effects were not observed when ACL fibroblasts were exposed to PBMCs with PPP or saline. Therefore, platelets may be essential for PBMCs to exert an anabolic effect on fibroblasts and may execute this effect by stimulating PBMCs to secrete IL-6 [51] and release their multiple growth factors. On the other hand, leukocytes in PRP also release inflammatory cytokines, including IL-1β, TNF-α, MMP-8, and oxygen-free radicals, that cause catabolic effects on cells and damaged tissues[53]. Whether the beneficial effects of leukocytes in PRP surpass the detrimental effects still remains a matter of debate, and additional studies are needed to prove or disprove this argument. Regardless, it is suggested that in clinical applications PRP without leukocytes should be used for tendon and ligament repair because of the potential complications from inflammatory cytokines and toxic molecules released by leukocytes [54].

It should be noted that when activated, PRP is like a fibrin matrix, which incorporates platelets and facilitates platelet-derived growth factors release at the site of injury to improve tendon repair. Fibrin also contributes to the formation of a flexible network supporting cytokine enmeshment and cellular migration[55]. However, higher concentrations of fibrin is reported to decrease fibroblast proliferation and biosynthetic activity[56].

Molecular components

Growth factors

It is beyond doubt that growth factors released by activated platelets in PRP play a crucial role in enhancing TLI healing. Many studies have elucidated the functions of these growth factors[1,17,23,44], including PDGF, TGF-β, VEGF, IGF, HGF, EGF, and bFGF. All these growth factors are also markedly up-regulated following TLIs and are active during multiple phases of the healing process[23,57,58].

PDGF is released by the degranulating platelets at the time of injury, and peaks shortly after tendon damage[59]. It plays multiple roles in the TLI healing process as a whole, including the inflammatory, proliferation, and remodeling phases. For example, it regulates chemotaxis, angiogenesis, mitogenesis of fibroblasts, as well as macrophage activation; it also stimulates the production of other growth factors, such as IGF-I, TGF-β1, and VEGF[60,61]. In fact, application of PDGF alone has been demonstrated to improve the strength and stiffness of treated ligaments[62].

TGF-β exists in platelets, macrophages, fibroblasts, and other cell types, with platelets being the major producers of TGF-β[25,59]. Similar to PDGF, TGF-β is active throughout the whole process of tendon healing[23] and exerts various effects, such as fibroblast stimulation, macrophage recruitment, cell proliferation, and collagen production[63]. Although TGF-β enhances tendon healing, it also contributes to the formation of uncontrolled excessive scarring[25]. Additional studies are needed to find the optimal levels of TGF-β to form normal tendon tissue, but not result in disorganized scar formation.

VEGF is a powerful stimulator of angiogenesis, and is produced in high levels only at the end of the inflammatory phase[64]. A number of studies have shown that VEGF improves revascularization and the biomechanical properties of autografts and allografts after ACL reconstruction[65-68]. Despite VEGF's widely-reported positive effects on tendon and ligament healing, it also causes some adverse effects. VEGF application has been reported to temporarily decrease the strength of grafted tendons after ACL reconstruction[69]. One presumed reason for this is that the newly formed vessels and infiltrated cells induced by VEGF act as "flaws," which decreases the mechanical properties of ACL grafts. Another plausible reason could be that enhanced production of matrix metalloproteinase (MMP) induced by VEGF directly "digests" the matrix in the graft[68].

IGF-I is an important mediator involved in all phases of wound healing. It stimulates fibroblast proliferation and migration, collagen gene expression and protein synthesis, as well as extracellular matrix synthesis both *in vitro* and *in vivo* on animal models[70,71]. In humans, local injection of IGF-I into the patellar tendon increases collagen synthesis[72]. Moreover, application of IGF-I to tendons improves the ultimate load[24,73].

EGF is important for the migration and proliferation of tendon/ligament cells[70,74], and collagen synthesis[75]. However, EGF also imparts negative effects on ligament cells and collagen production[76]. For example, the addition of EGF to ACL fibroblast cultures slightly decreases collagen production and the ratio of collagen type I to type III[70]. Therefore, the beneficial effects of EGF on TLI healing need further investigation.

bFGF, which is present in blood at very low concentrations[77], stimulates angiogenesis and regulates cell

proliferation and migration[78,79]. These stimulatory effects of bFGF are concentration-dependent: 2 ng/ml and 10 ng/ml lead to significantly better wound closure than the control, but a higher concentration of 50 ng/ml impairs wound closure[78]. It is presumed that bFGF at a higher concentration stimulates other low-affinity receptors and thereby impairs wound closure[80].

In addition to being an anti-inflammatory molecule[81], HGF is an angiogenic mediator, derived from both platelets and plasma[17,82]. It induces VEGF expression during inflammation and contributes to angiogenesis by participating in the proliferation and remodeling phases of tissue healing[83]. Local administration of HGF accelerates healing after medial collateral ligament (MCL) injury[84,85]. HGF also promotes tissue regeneration and reduces scar formation because of its antifibrotic property [86]. However, it is still a considerable challenge to promote recovery of injured tendons and ligaments without causing excessive scar formation.

It should be emphasized that while PRP contains all the growth factors mentioned above, its "collective" function in TLI healing is not the "sum" of the functions of individual growth factors.

Other factors

Besides the aforementioned growth factors, PRP contains a wide variety of less-emphasized molecular components such as PF-4, osteocalcin, osteonectin, fibrinogen, vitronectin, fibronectin, and thrombospondin-1, which are released from α-granules after platelet activation. These proteins also induce cellular proliferation, extracellular matrix formation, and collagen synthesis[44]. In addition, adenosine, serotonin, histamine, and calcium released from dense granules also play a role in tissue healing[31].

Furthermore, autologous thrombin in PRP has gradually attracted researchers' attention, as it is safer than bovine thrombin, which is frequently used to induce PRP gel formation in patients with tendon/ligament injury[87]. Bovine thrombin has been shown to induce antibodies against factor V, factor XI, and thrombin that can result in potentially life-threatening conditions[88]. Apart from converting fibrinogen into fibrin during clot formation, thrombin has been shown to directly stimulate mitogenesis, stimulate cells to release elaborate growth factors, and increase the mechanical strength of healed tendons[89,90].

In addition, PRP also contains multiple antimicrobial peptides[91] that contribute to the antimicrobial effect of PRP[92]. The antibacterial functions of autologous PRP have been a hot research topic in recent years[93-95].

Basic science studies on PRP

The majority of basic science studies using *in vitro* and *in vivo* models clearly demonstrate PRP's ability to accelerate the healing process of TLIs[15,29,40,96-99]. These findings shed new insights into the cellular and molecular mechanisms by which PRP enhances the healing of injured tendons and ligaments. It is known that repair of injured tendons and ligaments requires progenitor/stem cell recruitment and differentiation, fibroblast proliferation, collagen production, angiogenesis, and mechanical loading, which are discussed below.

Fibroblasts and collagen

The major proportion of cells in tendons and ligaments are fibroblasts, and the major extracellular component is collagen. A number of basic studies have demonstrated that PRP can increase both the number of cells and the cellular component by enhancing fibroblast proliferation and collagen production, and thus strengthen healed tendons and ligaments [15,17,100,101]. Application of the PRP releasate to human tendon cells in culture stimulated cell proliferation [17] and when healthy tendon and ligament tissue explants were cultured in medium containing PRP, there was an improvement in both COL1A1 expression and the ratio of COL1A1:COL3A1[101]. In addition, PRP injection into the Achilles tendons of rats improved not only collagen production in tendons, but also their tensile strength[102].

Tendon stem/progenitor cells

Besides fibroblasts, tendons contain another important cell population: tendon stem/progenitor cells (TSC)[15,103,104]. TSCs play a vital role in tendon maintenance and repair due to their ability to self-renew and differentiate into tenocytes[2]. PRP releasate induces differentiation of TSCs into tenocytes, which proliferate quickly and produce abundant collagen[15].

Circulation-derived stem cells

Apart from its effects on local TSCs and fibroblasts, PRP also has a strong potential to recruit circulation-derived stem cells. Local injections of PRP into rat patellar tendons increases the migration of circulation-derived stem cells, which are more proliferative and produce more collagen type I and type III than the control group[29].

Angiogenesis

The angiogenic effect of PRP is attributed to the presence of endothelial growth factor (EGF) in the endothelial cells, which are primarily responsible for new blood vessel growth, because neutralization of EGF in PRP causes a significant decrease in endothelial cells proliferation[105]. PRP injections into a mouse leg ischemia model for angiogenic evaluation increased the number of newly-formed blood vessels

and the blood reperfusion levels of ischemic tissues when compared to the control groups[106].

Mechanical loading

Mechanical loads greatly influence tenocyte differentiation and proliferation. Many studies have shown that mechanical loading enhances fibroblast proliferation, collagen synthesis, and collagen realignment, leading to increased tendon strength and accelerated tendon healing[2,29,107]. Appropriate mechanical loading is beneficial for the tendons because it induces differentiation of TSCs into tenocytes[15]. However, excessive mechanical loading induces differentiation of TSCs into non-tenocyte lineages such as adipocytes, chondrocytes, and osteocytes, in addition to tenocytes[15]. One likely reason for this aberrant differentiation, which could lead to tendinopathy, is an increase in PGE_2 levels induced by mechanical overloading. It has been shown that PGE_2 decreases TSCs proliferation and collagen production and induces both adipogenesis and osteogenesis *in vitro*[15].

Age-dependency

The effects of PRP are also age-dependent. Application of PRP obtained from immature/young patients significantly increased both cell migration and proliferation of human ACL fibroblasts than PRP from mature individuals[56]. This is likely due to the higher quantities of healing-capable stem cells known to exist in immature or young humans than older ones, with levels ranging from 1/100,000 in a teenager, 1/250,000 in a 35 year old, 1/400,000 in a 50 year old, and 1/1,200,000 in a 80 year old[59,108]. Therefore, it may be postulated that young patients would respond more favorably to treatment with PRP.

Clinical applications of PRP

Clinical applications of PRP for TLIs mainly focus on Achilles tendon injury, rotator cuff repair, patellar and elbow tendinosis, and anterior cruciate ligament (ACL) reconstruction. Although standardized treatment guidelines for the use of PRP are limited [Platelet Rich Plasma (PRP) Guidelines, 2010, 2011 by the International Cellular Medical Society] and large-sample, high-quality scientific studies are still not available to support its use, PRP treatment is increasingly utilized in sport medicine. More than 86,000 athletes with acute and chronic tendon, ligament, and muscle injuries are reported to be treated with PRP annually in the United States and some European countries[77].

Overall, PRP treatment has an excellent safety record in clinical applications. The adverse effects reported are minimal with only one patient having a synovial reaction around the ACL[109] and another patient reporting severe pain that lasted for 3 weeks[9]. However, these are common reactions, and the rate of pain after PRP injection was not higher than the rate in control group[110].

Treatment of injured tendons with PRP

Tendons with acute injury

Acute tendon injury has become a common problem in modern society, with spontaneous Achilles tendon ruptures occurring frequently among athletes[111] and active individuals. In general, surgical treatments have offered patients fairly good functional results, but like any other surgery, complications also occur during tendon surgery, including long recovery periods, high incidences of re-rupture, and wound infection[32,112].

In an effort to treat athletes with complete ruptures of the Achilles tendon, activated PRP was injected into the tendons during a surgical procedure[32]. Six athletes treated with PRP took significantly less time to improve their range of motion in the ankle, resume gentle running, and begin training activities when compared to control athletes who had conventional surgical treatment. This was the first study to report on the efficacy of PRP in the treatment of injured tendons, but it is retrospective and has a limited sample size (a total of 12 athletes). Another study using PRP to treat acute tendon injury is a prospective randomized controlled trial[113]. However, in this study the ATTRS (Achilles tendon total rupture score) was lower in the PRP group, indicating that PRP had a detrimental effect on the repair of acutely injured tendons. It should be noted that, in this study, all postoperative patients used a cast for 7 weeks, which could have prevented the mechanical stimulation of tendons that is necessary for tendon healing[114]. Moreover, an excessive concentration of platelets in PRP (17 times that in whole blood) might have been a factor that hindered tendon healing.

Tendons with chronic injury

In clinical sports medicine PRP is widely used for the treatment of chronic tendon injuries, particularly elbow-, Achilles-, and patellar-tendinopathy. However, the reported effects of PRP on these conditions vary among different studies. While clinical results have consistently shown the effectiveness of PRP in the treatment of chronic elbow tendinopathy[115], studies on its efficacy in the treatment of chronic Achilles- and patellar-tendinopathy have reported conflicting results[116-119].

Elbow tendinopathy

As mentioned above, PRP applications have been consistently effective in the treatment of elbow tendinopathy. In a number of RCT studies, PRP injec-

tions significantly improved outcome measure scores, including VAS (Visual Analog Scale, one of the most commonly used measures of pain intensity) and DASH (Disabilities of the Arm, Shoulder, and Hand) scores in the experimental groups compared to the control groups, which were injected with either whole blood, saline, or corticosteroid[13,31,115,120-122]. In one study that compared PRP with corticosteroid injection, the results showed significantly better VAS and DASH scores in the PRP group than in the corticosteroid group, which showed better scores initially followed by a decline, whereas the PRP group showed progressive improvement[121].

Achilles tendinopathy

In a prospective case series study where PRP was used to treat chronic Achilles tendinopathy, significant improvements were reported in clinical scores and ultrasonographic assessment[116]. In follow-up visits 18 months after treatment, AOFAS (American Orthopedic Foot and Ankle Society) scale scores (a scoring system to assess and monitor patients after foot and ankle surgery) improved from 55 to 96 points ($P < 0.01$), while the VISA-A (Victorian Institute of Sport Assessment-Achilles) scale scores (a scoring system to assess patients with Achilles tendinopathy, with a score of 24 for very poor condition to a score of 90 for excellent condition) improved from 24 to 96 ($P < 0.01$). Similar results were also reported 3 months after PRP treatment, with AOFAS scores increasing from 34 to 92[117]. In contrast, a growing number of studies showed no difference in function, pain, or healing tendon structure after PRP treatment[118,119]. In the stratified, block-randomized, double-blind, placebo-controlled trial study by de Vos et al.[119], VISA-A scores improved in both the PRP injection group and the saline injection group, with no significant difference between the groups. Another Randomized Control Trial (RCT) in the same medical center reported similar results, with improved VISA-A scores and ultrasonographic tendon structures found in both PRP and control groups, without a significant difference between the groups[118]. One criticism of these two studies is that both the PRP and saline groups were given additional eccentric exercises, which may have overshadowed the possible beneficial effects of PRP[123].

Patellar tendinopathy

Studies on the treatment of human patellar tendinopathy with PRP have become more frequent only recently [8,9,124-127]. In a case series, PRP was injected three times every 15 days to treat patellar tendinopathy and patients showed significant improvements in Tegner activity scale (a score of activity level to assess patients with ligament injuries), VAS, and SF-36 scores (a score system to assess patients' physical and mental health, pain, social function, etc.) at 6-month follow-up visits[9]. The same research group also conducted a prospective RCT and found similar results, with greater improvement in sport activity level in the PRP group (percentage improvement, 39 ± 22%) compared to the control group (20 ± 27%) ($P = 0.048$)[124].

Treatment of injured ligaments with PRP

Clinical studies of PRP treatments for injured ligaments mainly focus on surgical anterior cruciate ligament (ACL) reconstruction, primarily because of the high incidence of ACL rupture in the United States which is 400,000 per annum[51].

In a prospective, randomized, double-blind clinical trial[128], contrast-enhanced magnetic resonance imaging (MRI) was used to evaluate the revascularization process in the osteo-ligamentous interface zone, which showed significantly higher vascularization in the PRP group (0.33 ± 0.09) compared to the control group (0.16 ± 0.09, $P < 0.001$). The results from another RCT performed by the same group[129] reported improved knee stability after ACL reconstruction and local application of PRP. In contrast, an increasing number of studies have reported no benefits of PRP application. In these RCTs, no significant improvement was observed in graft MRIs, clinical or functional scores after PRP application[109,130,131]. The reason for the failure of PRP to confer any additional benefit over ACL reconstruction procedures was considered to be due to the presence of intra-articular plasmin in the post-traumatic joint[51], which degrades fibrin within PRP, thus degrading it before it can exert its effect[132].

Concluding remarks

A large number of studies have demonstrated the beneficial effects of PRP on the healing of tendons and ligaments at the molecular, cellular, animal, and human trail levels, but the efficacy of PRP treatment for TLIs is still controversial in orthopaedics and sports medicine fields. This situation may be attributed to the complexity and diversity of PRP preparations used in these studies. Therefore, there is a pressing need to study the efficacy of PRP in the treatment of TLIs through basic science studies, especially given that clinical practices of PRP treatment are far ahead[133,134]. For this, several factors require optimization, including compositional variations in PRP preparations and the dosage used in treatments, among others. In addition, the same PRP treatment regimen, mainly in the form of PRP injections, is applied to all TLIs particularly tendinopathies. Because tendinopathy is a spectrum of tendon disorders[135], the "one-size-fits-all" approach, generally consisting of PRP injections alone, may not be effective for all cases of tendinopathies, which can range from early stage, which is mainly inflammatory with damage to tendon matrices[136], to advanced stage, which is mainly degenerative with extensive formation

of fatty tissues, cartilage-like tissues, and bone-like tissues (calcification), either alone or in combination, within tendon lesions[137]. Another layer of complexity is added by the age, gender, disease history, and treatment history of individuals in the studies. Lastly, subjective evaluation of PRP treatment effects, such as pain and functional scores from patients, cannot be completely avoided[118,119]. These limitations may be best addressed by studying outcomes of this treatment on well-defined animal models of tendinopathy.

The healing process of TLIs is different from the healing of other tissues, and the biochemical and mechanical properties of healed tendons and ligaments never regain the properties of normal/uninjured tendons and ligaments due to scar formation after the repair of an injury. Therefore, the ultimate goals of TLI research should focus on using PRP to prevent scar formation, thus resulting in the recovery of normal structure and biomechanics of healed tendons and ligaments.

In particular, some special characteristics of PRP should be taken into account. PRP contains a mixture of anabolic and catabolic mediators. These mediators may change, depending on the physiological and pathological states of the person and injury sites where PRP is delivered. It has been shown that tumor-conditioned platelets, which were derived from animals with highly-angiogenic tumors, exhibited enhanced neovascularization[138]. Additionally, some evidence suggests that the activated receptors in platelets may facilitate a selective release of growth factors[139,140]. Therefore, how to trigger the release of only beneficial cytokines from PRP may be an important direction for future research.

Complex relationships among various molecules may be studied via the neutralization of individual or multiple growth factors. Although there are some studies in this direction[25,141], these relationships are still far from being clearly understood.

Moreover, when examining differing efficacies of PRP in TLI repairs, it should also be noted that tendons or ligaments at different sites vary in their potential for spontaneous healing. For example, ACLs are known to have poor healing potential compared to MCLs[142,143]. As a result, injury to ACLs often necessitates surgical intervention, whereas injured MCLs often require only conservative treatments in clinics.

Lastly, the effects of rehabilitation protocols after PRP treatment must also be established. Many controversies exist around these effects. Tendon healing in rats with post-operative mobilization was shown to be significantly better than the group that remained immobilized after operation[114,144]. Theoretically, mechanical stimulation should be given to patients once sufficient quantities of fibroblast and collagen are formed and are capable of transferring at least some of the mechanical load[89]. Although mechanical stimulation helps restore gliding function, enhances tendon/ligament strength and morphological restoration[111,145-147], patients who undergo rupture repair operations are often immobilized in clinical conditions because of fear of re-rupture. Therefore, the need to provide appropriate and timely loading must be weighed against the need to cast until fibroblasts and extracellular matrix have regenerated enough to bear the loading.

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