CHAPTER 11

Tendinopathy and its Treatment: the Rationale and Pitfalls in the Clinical Application of PRP

AUTHORS

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SUMMARY

Chronic tendon injuries or tendinopathy are prevalent among athletes and non-athletes worldwide. However, current treatment of tendinopathy in clinical settings is largely palliative. Platelet-rich plasma (PRP) is now a popular option to treat tendinopathy in orthopaedic surgery and sport medicine although some clinical studies cast doubt on the efficacy of PRP treatment for tendinopathy. Based on the findings from most basic science studies it seems clear that PRP can reduce pain and promote healing in tendinopathic tendons. However, the application of PRP treatment in clinics needs optimization. Currently, PRP treatments use a "one-size-fits-all" approach where a predetermined dose of a PRP preparation is used to treat tendon injuries in patients regardless of the conditions of tendon injury or patient's age, gender or treatment history. This "one-size-fits-all" approach would diminish the efficacy of PRP treatment and as a result, would create contradicting clinical outcomes. Thus, a "personalized approach" is necessary where PRP- and patient-related factors should be taken into account prior to PRP treatment. These may include selecting an appropriate PRP and also considering patients' age, gender, injury type, disease history, pre-injury activity level and post-treatment rehabilitation protocol. It is expected that such "personalized medicine" approach would enhance the efficacy of PRP treatment in clinics.

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1. TENDINOPATHY

Tendons are connective tissues that link muscles to bones, and are mainly made of cells, collagen fibers and small amounts of proteoglycans that form the tendon extracellular matrix (ECM). The primary function of tendons is to transmit muscular forces to bones, enabling joint movements. Therefore, tendons like patellar and Achilles tendons are in general subject to large mechanical loads. Many studies have indicated that excess mechanical loading may induce chronic tendon injury or tendinopathy[1]. More recent studies have implicated tendon stem/progenitor cells (TSCs) in the mechanical loading-induced degenerative tendinopathy[2]. When exposed to mechanical overloading, TSCs were shown to differentiate not only into tenocytes, the dominant resident cells in the tendons, but also into non-tenocytes (adipocytes, chondrocytes and osteoblasts)[3] that may disrupt tendon structure and integrity.

Moreover, mechanical overloading is also implicated in inducing sterile tendon inflammation commonly observed in tendinopathic tendons[1]. Sterile inflammation is characterized by the presence of PGE2, an inflammatory mediator, in injured tendons. Studies have reported higher PGE2 levels in the Achilles tendons of humans subjected to mechanical loads in vivo[4] and in human tendon cells after mechanical loading in vitro[5]. Similar effects were also observed in animal models where intensive treadmill running increased PGE2 in the patellar and Achilles tendons of mice in vivo[6]. While low levels of PGE2 may increase tenocyte gene expression, high levels of PGE2 in the tendon may increase non-tenocyte gene expression and result in the formation of non-tendinous tissues by causing differentiation of TSCs into non-tenocytes[6] thus leading to tendinopathy (Fig. 1). The high levels of PGE2 production in injured tendons is the rationale behind using non-steroid anti-inflammatory drugs (NSAIDs) to treat tendinopathy; NSAIDs inhibit COX, which catalyze the conversion of arachidonic acids to PGE2[7]. Thus, inhibiting COX by NSAIDs reduces the levels of PGE2 in injured tissues like tendons thereby decreasing inflammation.

High levels of prostaglandin E2 (PGE2) may induce the development of tendinopathy

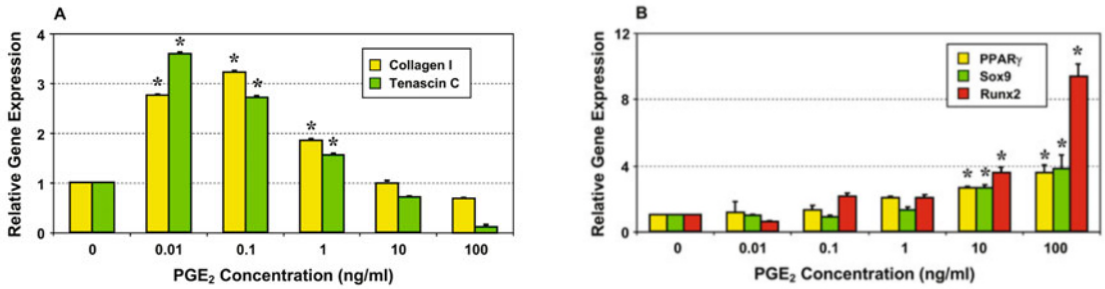

FIG. 1
(A) In tendon cells in vitro, PGE2 modulates the expression of tenocyte and non-tenocyte related genes in a dose-dependent manner. Specifically, at 0.01 ng/ml concentration, PGE2 induces a high level of expression of collagen type I (Collagen I) and Tenascin C, two tenocyte-related genes. (B) On the other hand, high concentrations of PGE2 increase non-tenocyte related genes, PPARγ (fat tissue marker, Sox9 (cartilage marker) and Runx2 (bone marker).

* $P < 0.05$ compared to 0ng/ml PGE2 concentration. For full description of the findings, refer to the paper[78].

Besides mechanical overloading, another common but less addressed causative factor of tendinopathy is aging, which affects a majority of the aging population. Typically, aging reduces the quality of human tendons by decreasing tendon cell numbers, protein synthesis and water content. Apart from these, non-tendinous tissues such as lipid deposition, proteoglycan accumulation, and calcification that are often observed in tendinopathic tendons[8] are also noticed in aging tendons. Using a mouse model, our study showed that tendons in aging mice (9 months old) had higher amount of lipids, proteoglycans and calcium deposits in comparison with young 2.5 months old mice[9]. TSCs isolated from aging mice also expressed higher amounts of non-tenocyte genes, LPL, Sox9 and Runx2[9] that have the potential to induce the development of lipids, proteoglycans and calcium, respectively. Moreover, the quality of TSCs in aging mice is evidently poor with lower proliferation rate and reduced expression of stem cell markers (Oct-4, Sca-1, and SEEA-1) (Fig. 2)[9], which may likely impair the healing of tendinopathic tendons because TSCs are necessary for the maintenance of intact tendons and repair of injured tendons by differentiating into tenocytes to replace cells and matrix proteins lost due to injury.

Aging causes tendinopathy by deteriorating tendon stem cells

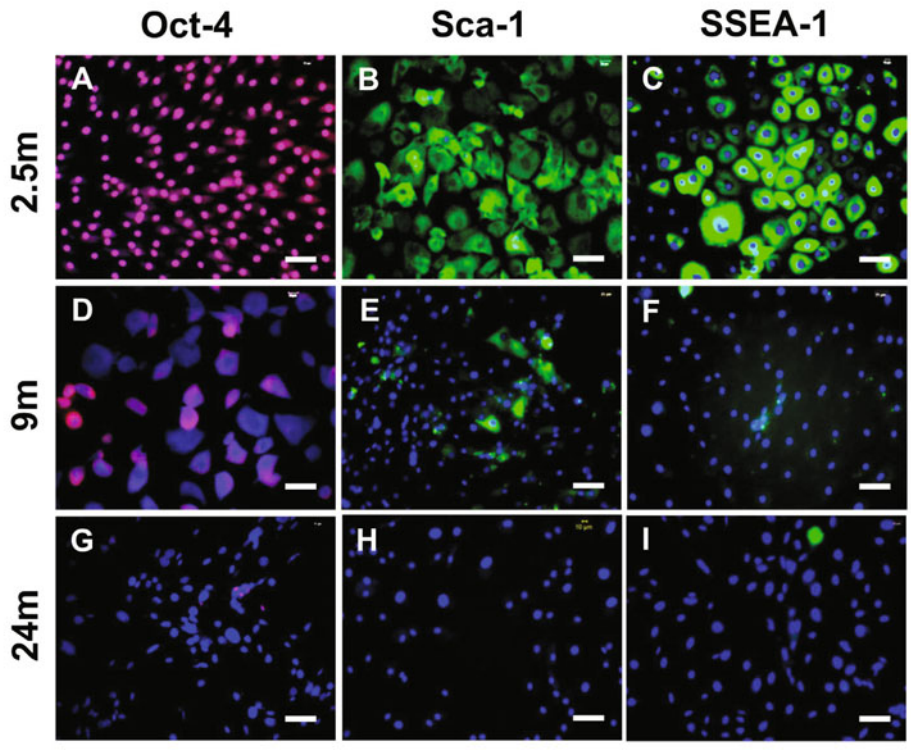

FIG. 2
Almost all tendon stem/progenitor cells (TSCs) from young, 2.5 months old mice express robust amounts of stem cell markers, Oct-4 (pink, A), Sca-1 (green, B) and SSEA-1 (green, C). But in 9 months old mice, fewer cells express these stem cell markers (D-F) and in 24 months old mice, none of the cells express these markers (G-I). Bar – 50μm. For full description of the results regarding the effects of aging on the quality and number of TSCs in mice, see the original paper [9].

2. TRADITIONAL TENDINOPATHY TREATMENT

Tendon and ligament injuries are one of the most prevalent musculoskeletal problems with millions of patients treated in orthopaedic clinics in the United States every year. Among the several tendons in a body, patellar, Achilles, and rotator cuff are the three tendon types that are more susceptible to injury than others due to mechanical overloading/overuse. Patients with tendinopathy experience inflammation and pain, and in many cases tendon rupture due to degenerative changes in the tendon. Currently, these patients receive only palliative care in clinics where pain and inflammation are treated mostly with NSAIDs, corticosteroids, physical therapy, shock wave therapy, or even rest with the last option being surgery. Although NSAIDs offer a short term pain relief, they have a negative impact on the structure of injured tendons, and are also reported to cause serious side effects such as abdominal discomfort, and gastrointestinal, cardiovascular and renal problems[7]. Besides, the use of corticosteroids itself may lead to tendon rupture by decreasing tendon cell proliferation and inducing degenerative changes in the tendon[10]. The other modalities often yield inconsistent results. Thus, alternative treatment options are in need for the safe and effective treatment of tendinopathy.

3. PLATELET-RICH PLASMA (PRP) TREATMENT OF TENDINOPATHY

Currently, PRP therapy is widely used as a promising option for tendinopathy treatment in orthopaedic/sports medicine across the United States and other countries[11]. Intuitively, the use of PRP for healing tendon injuries is reasonable, because in the event of an injury, platelets are indeed the "first responders" and thus PRP treatment may mimic the natural wound healing process. The primary benefits of PRP are a reduction in tendon pain and improvement in tendon function, which have been reported by patients treated for a variety of tendinopathy such as patellar tendinopathy[12], Achilles tendinopathy[13], elbow tendinopathy[14], and chronic plantar fasciitis[15].

In the PRP treatment of tendinopathy, the main factor to be considered is the PRP composition. The major PRP components are as follows.

Platelets

Platelets are present in large amounts because PRP is prepared with blood drawn from patients by centrifuging at low speed so they are re-suspended in a small volume of plasma. Platelets are anucleate cytoplasmic fragments produced in the bone marrow by megakaryocytes[16]. In the event of an injury, they are also the first responder cells that migrate to the wounded site and are well-known to play an important role in tissue healing[17,18].

It has been recognized that tissue wound healing is driven by growth factors, which are present in high amounts in PRP[17,18]. The main growth factors in PRP include platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF). These growth factors have been shown to enhance tendon healing[17,18]. Depending on the platelet concentration in PRP, the amount of growth factors released into a treated site may vary. While the rationale behind using PRP is to deliver concentrated amounts of growth factor-containing platelets to heal an injured tissue, studies have shown that very high levels of platelets may not be useful to treat tendon injuries[19] because they do not induce tendon cell proliferation[20]. In fact, the platelet concentration in most clinical and basic science studies is only 3-5-fold higher than in whole blood. At this concentration, PRP has been shown to increase the number of tendon cells[20,21], enhance collagen synthesis[20-22], improve fiber arrangement[23], and enhance tendon strength[24]. However, when the platelet concentration was more than 10-

fold higher than in whole blood, improvement in tendon function was not evident in patients with Achilles tendon ruptures[19]. Therefore, it is pertinent to regulate the platelet concentration in PRP, which are currently prepared using commercial kits in clinical settings.

Plasma

The majority of plasma is made of water (90%) and other components such as proteins. Plasma is necessary for the fluidity of the blood, and allows the flow of cells and other factors around the body.

Leukocytes

Another important component in PRP is the leukocyte, which has gained attention only recently in basic PRP research and clinical applications. Depending on the preparation method, PRP may contain a few or high amounts of leukocytes; PRP with no or only a few leukocytes are termed pure-PRP or P-PRP and those with high amounts of leukocytes are called leukocyte containing or leukocyte rich PRP both referred to as L-PRP.

Fibrinogen

PRP also contains abundant fibrinogen, which is a soluble plasma protein. When PRP is activated with thrombin or calcium chloride, fibrinogen is converted into polymeric fibrin, which forms a gel-like substance. In this gel form, PRP can act as a conducive bio-scaffold/matrix that facilitates the interaction between the components of PRP, tendon tissue and other migrating stem cells that are recruited to the healing site[25]. Because of this, PRP can also be used as an excellent carrier of cells or a bio-compound in wound repairs using tissue engineering approaches. For example, PRP was used as a carrier to deliver autologous adipose derived-stem cells (ASCs) to repair Achilles tendon defects in rabbits[26]. Our own studies have also used PRP as an effective carrier of kartogenin, a small bio-compound, to promote fibrocartilage formation in the tendon-bone interface[27].

Because PRP is a natural source and has a unique composition, it has a number of advantages that promote its use to treat tendon injuries.

1. PRP is safe because it is an autologous product derived from a patients' own blood and also contains physiological proportions of the growth factors;
2. PRP preparation in orthopaedic clinics is straightforward with many commercial kits available;
3. PRP can be easily applied via injections in clinical settings and therefore it is non-surgical and allows athletes to quickly return to sport activities;
4. PRP serves as a reservoir of numerous growth factors that can enhance tendon healing; and its fibrin gel can be used as a natural scaffold that is conducive to tendon healing; and
5. PRP can function as an anti-inflammatory "drug" to reduce tendon inflammation and hence pain. Such beneficial effects of PRP are better than the currently used steroid treatment that induces serious side effects.

4. CLINICAL STUDIES ON PRP

The findings of laboratory science become fruitful when validated by clinical studies such as randomized clinical trials (RCTs), which are considered as gold standards for clinical studies. Thus, a number of RCTs have been conducted to determine the efficacy of PRP to heal tendinopathic tendons with many reporting the beneficial effects of PRP in patients. For instance, significant pain reduction was observed in an RCT that included 14 patients with chronic lateral elbow epicondylitis 6 weeks after PRP treatment when compared to the 14 patients treated with autologous blood[28]. Similarly, pain reduction was realized 6 months after PRP treatment of patellar tendinopathy in an RCT with 12 patients[12]. In yet another larger double-blind, prospective, multicenter RCT, PRP treatment of chronic tennis elbow in 116 patients improved

pain and function 6 months after treatment when compared to an active control group with 114 participants[29]. Furthermore, PRP also caused long-term pain-reduction effects. In a double-blind RCT that included 100 patients with chronic lateral epicondylitis, PRP treatment significantly reduced pain and improved elbow function in the 51 patients in this group in 1-year and 2-year follow-up studies when compared to corticosteroid injections given to 49 patients[14]. Besides, PRP has been used in conjunction with stem cells to improve PRP treatment efficacy. An animal RCT administered a combination of adipose derived mesenchymal stem cells and PRP, and found that the combination curtailed lesion progression, enhanced collagen fiber organization and decreased the presence of inflammatory cells[30]. Other clinical studies have also reported beneficial effects of PRP in patients with chronic plantar fasciitis[15], elbow tendinopathy[14,28,29], chronic Achilles tendinopathy[13] and patellar tendinopathy[12].

Despite the encouraging findings in these RCTs, others have reported the opposite results of PRP treatment; i.e. PRP did not produce beneficial effects when compared to the control. In fact, PRP did not significantly improve symptoms in patients (n = 27) with chronic Achilles tendinopathy when compared to the saline treated group up to 1 year after the treatment[19]. Similar results were also obtained in another RCT where PRP was used to treat rotator cuff tendinopathy in 20 patients; PRP treatment was similar to the saline treated group after a 1 year follow-up[31]. However, the study by Schepull et al.[19] used PRP with unusually high platelet concentration; 10-fold higher than in whole blood, while basic science studies use PRP with only 3-5 fold higher platelets level, which showed beneficial effects at the cellular and molecular levels[20,21]. Similarly, treatment of elbow tendinopathy with L-PRP or autologous whole blood yielded similar pain scores in 76 patients (n=38 per group) in a one year follow-up study[32]. Interestingly, using L-PRP to treat patellar tendinopathy in 10 patients in an RCT significantly improved pain and tendon function (VISA scores) in the PRP treated group when compared to the dry needling treatment group (n = 13) at 12 weeks[33]. However, at 26 weeks, the improved treatment effects of PRP were slightly lower than the dry needling treatment[33].

Thus, it is clear that while PRP appears to exert beneficial effects, such as reducing pain and improving tendon function, in some clinical trials, others reported no effects thus creating controversies in the efficacy of PRP to treat tendinopathy. Since RCTs are the gold standard for clinical studies, results from RCTs are considered highly relevant and important for judging the effectiveness of PRP treatment for tendinopathy. But it is important to note that while positive results from RCTs provide evidence for the efficacy of PRP among human participants, the negative results should be interpreted with caution taking into consideration the common factors that may lower PRP efficacy. A common weakness with RCTs is the small number of participants. In the RCTs mentioned above, except except for some with a few hundred participants the remaining had relatively small sample size (n < 25). Considering extreme variability among humans receiving the treatments, such a small sample size could reduce the statistical power such that the treatment effects of PRP may not be detected.

Another variable in the clinical trials of PRP treatment is the variations in PRP composition in the different preparations that may contribute to the controversy surrounding PRP efficacy. More importantly, the responses of study participants to PRP treatments may vary greatly and may not qualify to score as a positive effect. Conducting an RCT is highly complex where many factors such as age, gender, disease/treatment history and patient management protocols can affect the outcome. However, before evaluating these variables it is essential to understand whether there is any scientific basis for the use of PRP to treat tendinopathy. Below, we provide an analysis of basic science studies performed on cell (in vitro) and animal (in vivo) models under well-controlled conditions.

5. *IN VITRO* STUDIES OF PRP

Because in vitro studies are conducted in well-controlled experimental conditions it is possible to measure specific treatment effects of PRP. Below are some cellular events that are influenced by PRP.

Tendon cell proliferation

Studies have shown that PRP treatment of tendon cells in vitro increased their proliferation significantly in a dose-dependent manner[21,34]. However, a 10-fold higher platelet concentration in PRP did not benefit tendon healing[19] suggesting that a high concentration of platelets in PRP may not promote cell proliferation. Therefore, it is critical to use the optimal concentration of PRP (3-5-fold higher than in whole blood) to induce tendon cell proliferation[20,21]. Not only can PRP increase the proliferation of tendon cells it can also increase the proliferation of mesenchymal stem cells (MSCs), bone-marrow stem cells (BMSCs) and adipose derived stem cells (ADSCs) that may migrate into the injury site to promote tendon healing[24,34].

TSC differentiation into tenocytes

Like other adult stem cells, TSCs are also multipotent; i.e., they have the ability to differentiate into multiple cell types in the presence of appropriate induction factors. In the presence of PRP, rabbit TSCs differentiated into tenocytes but not non-tenocytes[20,21]. Moreover, PRP also increased the expression of tenocyte related genes, scleraxis and tenascin C[35], and matrix proteins, COMP, decorin and tenascin-C in tendon cells[36].The newly formed tenocytes were also active because they upregulated total collagen production[21], specifically collagen I and III[20].

Anabolic and catabolic effects of PRP

Collagen is the most abundant protein in the tendon ECM and many studies have shown that PRP can significantly promote the expression of collagen type I[20,22]. Specifically, TGF-β increased collagen I synthesis in tendon cells in vitro[37]. So the anabolic effects caused by PRP treatment on cells may explain why PRP can promote healing of tendinopathic tendons. Moreover, our in vitro study showed that PRP increased collagen production and did not induce non-tenocyte differentiation of TSCs into chondrocytes, adipocytes, or osteocytes that may lead to degenerative changes in the tendon[21]. This finding suggests that PRP treatment does not increase the risk of non-tendinous tissue formation in tendons that may compromise tendon structure and function. The main components in PRP that cause catabolic effects are the leukocytes. While leukocytes can be beneficial because they can promote chemotaxis, cell proliferation and differentiation[38], they can also be detrimental because they release pro-inflammatory cytokines such as interleukin-1 β (IL-1β) and IL-6, tumor necrosis factor- α (TNF-α) and reactive oxygen species that can induce inflammation thereby exacerbating the tissue injury[20]. In rabbit TSCs cultured in vitro, L-PRP increased the levels of inflammatory and catabolic proteins, IL-1β, IL-6 and PGE2, and matrix metalloproteinase (MMP)-1, MMP-13 while P-PRP increased anabolic proteins including alpha-smooth muscle actin (α-SMA), and collagen types I and III[20]. Similar differential effects were also observed in chondrocytes; L-PRP induced catabolism while P-PRP induced anabolism by increasing collagen type II and aggrecan expression[39]. Interestingly, leukocytes in PRP did not decrease tendon cell proliferation in vitro[20]. While platelets increase collagen type I expression, leukocytes promote collagen type III expression[36]. This information is of high value because a high collagen type I/collagen type III ratio is expected in normal healthy tendons while the reverse with high collagen type III/collagen type I ratio indicates injured tendons or tendons with scar formation[40]. Besides, L-PRP also increased MMP-1 and MMP-3 in tendon cells[20,36] while PRP without leukocytes decreased these MMP levels[20,22]. In addition, platelets increase the tendon matrix proteins, COMP and decorin, while leukocytes exert the opposite effects[36]. Thus, leukocytes mostly induce catabolic effects on tendons and tendon cells. Considering the above, it may be appropriate to use P-PRP or PRP with a low level of leukocytes in the clinical treatments of tendinopathy.

Anti-inflammatory effects of PRP

The popularity of PRP is primarily due to its ability to reduce pain and inflammation in tendinopathy patients in clinical trials. However, the mechanisms of these effects were only recently discovered. In an in vitro study conducted in our laboratory, PRP reduced the levels of the pro-inflammatory mediators, COX-1, COX-2 and PGE2 in TSCs. Similar effects were obtained under the same conditions when the cells were treated with HGF. Moreover, in a series of in vivo experiments anti-HGF antibodies reversed these effects; i.e., COX-1, COX-2 and PGE2 levels were not lowered in the presence of anti-HGF antibodies indicating that HGF, in part, exerts the anti-inflammatory effects of PRP (Fig. 3)[41]. In a previous study, HGF treatment also decreased the production of the pro-inflammatory cytokine, IL-6, but increased the anti-inflammatory cytokine, IL-10[42].

Combining PRP with other approaches

As mentioned above, once activated, PRP forms a gel; therefore, PRP can be combined with other tissue engineering modalities by mixing stem cells with PRP gel to treat tendinopathy. Combining PRP with BMSCs or TSCs to treat tendon wound healing induced synergistic effects[43]. Moreover, adding PRP to dexamethasone (corticosteroid) or ciprofloxacin treatment of human TSCs suppressed the non-tenocyte differentiation of cells and also reversed the reduction in cell senescence and cell death, induced respectively by the two drugs[44]. Similarly, a decrease in human tenocyte viability induced by methylprednisolone (steroid) was reversed by the addition of PRP[45]. Thus, PRP can serve as a better alternative to steroid treatments for the treatment of tendinopathy and may improve clinical outcome when combined with drugs to treat tendinopathy.

HGF in PRP is an anti-inflammatory factor

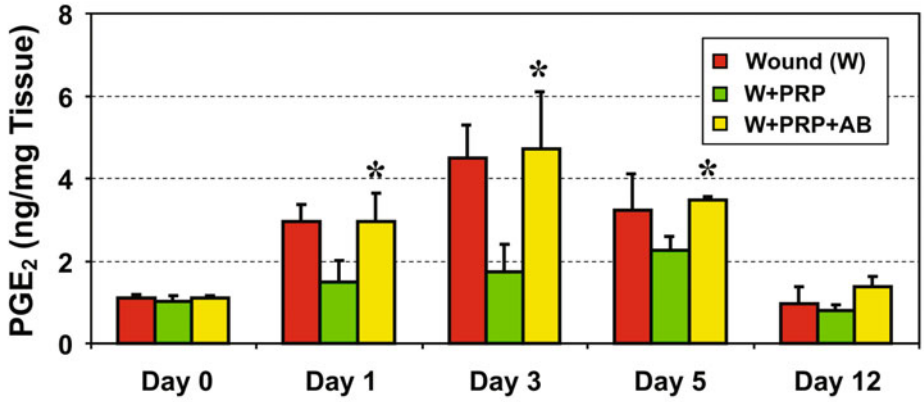

FIG. 3
In wounded mouse tendons, the concentration of PGE2, a tendon inflammation marker, increases on days 1-5 after wounding. However, treatment of the wounded area with PRP injection significantly reduces the levels of PGE2. In contrast, injecting HGF antibody along with PRP completely negates the PRP effects and as a result, PGE2 levels return to the levels in wounded tendons. AB, anti-HGF antibody.

* $P < 0.05$ when compared to the respective values on day 0. For complete results on the anti-inflammatory properties of PRP, see the original paper [41].

6. *IN VIVO* STUDIES OF PRP

In vivo studies have also shown the positive influence of PRP on tendon wound healing.

Intra-tendinous injections of PRP to treat tendinopathy in rat patellar and Achilles tendons increased joint mobilization and improved tendon fiber organization 25 days after treatment[46]. PRP gel injections into freshly ruptured patellar tendons also significantly increased the mechanical properties of tendons including stiffness, load at failure and ultimate stress over the saline treated control only 2 weeks after the treatment[47]. PRP also induced better cell orientation and tissue maturation in addition to increasing the expression of IGF-I in healed tendons[48]. Furthermore, individual growth factors including IGF-I[49], PDGF[50], FGF[51] or TGF-β[52] in PRP were demonstrated to increase cell proliferation and collagen production in tendon explants or when injected into horse, rabbit or rat tendons. Besides, combining platelet gel with a collagen implant effectively healed a rabbit Achilles tendon defect[53] and administering PRP with low-level laser therapy increased collagen type I and enhanced regeneration of the tendon tissue[54]. Thus, a number of in vivo studies have demonstrated that PRP treatment can enhance the healing of tendinopathic tendons. Lastly, our own studies in a wounded mouse Achilles tendon model established that PRP's anti-inflammatory function was mediated via HGF by suppressing the levels of COX-1, COX-2, and PGE2 production. The anti-inflammatory effects of PRP is of clinical relevance because high levels of PGE2 have been shown to increase pain[55], decrease cell proliferation and collagen production[56], and induce non-tenocyte differentiation of TSCs[21] that caused degenerative changes in rabbit tendons[57]. Therefore, PRP's ability to reduce PGE2 production is expected to benefit the healing of injured tendons.

7. FACTORS THAT MAY CONTRIBUTE TO THE PRP CONTROVERSY

As described above, findings from basic science studies provide support for the use of PRP to treat tendinopathy because PRP can increase cell proliferation, induce high expression of anabolic proteins, induce tenocyte differentiation, decrease inflammation and thus pain, which is of high clinical relevance. However, findings from some clinical trials do not align with basic science studies thus creating the well-known controversies in the PRP treatment efficacy for tendinopathy. We believe that the major factors that attribute to the inconsistent results observed in clinical trials are PRP-related factors, patient-related factors and insufficiencies of many clinical trials.

8. PRP-RELATED FACTORS

PRP treatment efficacy can be affected by a number of PRP associated factors including PRP composition, platelet concentration, activation, and application methods among others.

PRP composition

In current clinical practices, PRP is mostly prepared using various commercial kits, which do not yield "one-type" of PRP with the same composition. In contrast, PRP prepared from the kits vary in their composition. For example, PRP prepared from the same blood sample using three different kits (MTF Cascade, Arteriocyte Magellan and Biomet GPS III)[58] or three different preparation methods (apheresis-derived platelets, buffy coat-derived platelets and tube method-derived platelets)[59] had the same platelet concentration but had variable amounts of leukocytes. The potential negative effects of using L-PRP on tendon healing have been mentioned above. Therefore, it is suggested that PRP without leukocytes or with only small number of the leu-

kocytes should be used to maximize anabolic effects of PRP on the healing of tendinopathic tendons thus enhancing treatment efficacy. Moreover, different commercial PRP preparation kits yield variable levels of growth factor. For instance, PRP prepared from MTF Cascade, Arteriocyte Magellan or Biomet GPS III PRP preparation systems varied widely in their PDGF and VEGF levels[58]. Therefore, optimization of PRP composition is critical to improve the PRP treatment efficacy in clinics.

Platelet concentration

The growth factors containing platelets vary considerably based on the type of kit used to prepare PRP. Platelet concentration could be 1- fold (Autolo Gel System, Secquire), 3-5-fold (Biomet GPS, Cell Saver Based Systems, Sorin Angel, Harvest Smart Pre BMC, Depuy Symphony, Arteriocyte Medical Magellan) or even 10-fold (GenesisCS) higher than in whole blood. Therefore, it is pertinent to choose the PRP preparation with optimal platelet levels because the presence of too many platelets may not benefit tendon wound healing. In at least one RCT, a 10-fold higher platelet concentration in PRP over that in whole blood did not improve treatment outcomes[19], and most clinical and basic science studies only use PRP with platelet concentrations 3-5-fold higher than in whole blood. At these levels, PRP has been shown to exert beneficial effects; decrease pain, increase tendon cell proliferation, and improve collagen synthesis and organization[20,21].

Activation of PRP

Regarding PRP activation there seems to be no consensus in the method used for activation, which is essential for the release of growth factors from the platelets[59]. Traditionally, both basic science studies and clinical trials have used thrombin or calcium chloride to activate PRP externally prior to treatment. However, non-activated PRP can also be used to treat tendinopathy because platelets can become activated when they come in contact with collagen in vivo[28]. Recently, platelets in PRP were shown to contain different components; specifically, pro- and anti-angiogenic components were shown to be released selectively after activation by using agonists to proteinase activated receptor-1 or -4 (PAR-1 or PAR-4)[60]. Further research on this topic is warranted.

These variations in PRP suggest the need for a universal PRP labeling system describing the PRP components present in the preparations used in clinical trials[18,61]. An example would be the use of PAW that includes Platelet concentration, Activation method and presence of White blood cells/ leukocytes on the label[18] or DEPA that describes the platelet Dose, Efficiency of production, PRP Purity and Activation on the label[61].

Lastly, the frequency of PRP treatment, and the mode of PRP application via injection or implantation of PRP gel should be studied more for optimization.

9. PATIENT-ASSOCIATED FACTORS

A number of variables related to patients also contribute to controversial PRP treatment outcomes. These may include patient's age, patient's gender, injury type, patient's disease and treatment history, and post-recovery plans.

Patient's age

Currently, there is no data from clinical trials that compared the efficacy of PRP treatment in young vs old patients. Almost all clinical trials conducted thus far (see above) have included patients both young and old with their ages ranging from 18 - 70 years. Thus, while PRP treatment for tendinopathy may work in young patients, it may not work in older patients. One biological reason for this is that with increased age, TSC number and quality decreases[9]. Specifically, it was also shown that aging decreased the numbers of human MSCs in the bone marrow[62]; moreover, the proliferation rate of some stem cells including ADSCs[63], BMSCs[63] and ACL-derived cells[64] were higher in young than in older animals. Furthermore, the differentiation

potential of TSCs from older animals were found to be abnormal; i.e., TSCs from aging mice or rats could aberrantly differentiate into non-tendon cell types including adipocytes, chondrocytes and osteocytes[9,65] that develop into fat, cartilage-like and bone-like tissue in tendons and lead to tendon degeneration, which is a characteristic feature of degenerative tendinopathy[8]. In addition, some growth factors, for example in equine PDGF, had higher concentrations of PRP derived from younger horses than from older ones[66]. Thus, the increased number of TSCs, the higher TSC proliferation rate and higher amounts of growth factors in young individuals may result in a better PRP treatment efficacy than in older individuals. More importantly, the ability of aging TSCs to differentiate into non-tenocytes indicates how this stem cell characteristic could decrease the PRP treatment efficacy in older patients. Therefore, the PRP treatment efficacy in aging patients is expected to be low and if a clinical trial includes both young and old patients, it is expected that variations in clinical outcome of PRP treatment would be huge, which effectively reduces the statistical power to reveal the PRP treatment effects.

Patient's gender

Similar to age, clinical trials have not investigated the variations in PRP treatment efficacy based on patient's gender. Most clinical trials group males and females together and include more males than females. However, indirect evidence points to the likelihood of gender impacting PRP treatment efficacy in clinical trials. For instance, the levels of all human growth factors particularly EGF, HGF, IGF-1 and PDGF were found to be higher in females than males[67]. Besides, viscoelastic properties of tendons[68], and the incidences of Achilles tendon ruptures[69] and wound healing[70] are known to differ between genders. Moreover, among females PRP treatment efficacy can vary significantly between menopausal and post-menopausal women because estrogen impacts VEGF[71] and IGF-1[72] expression and lower estrogen levels decrease tensile strength[73] and diminish wound healing response of dermal fibroblasts[74].

Post-treatment recovery

Rehabilitation exercises after PRP treatment may benefit patients as shown in an RCT where patients were allowed eccentric exercise after PRP treatment of lateral elbow epicondylitis[28]. Besides, moderate mechanical loading can reduce inflammation[75] and excessive mechanical loading can increase inflammation by increasing PGE2 production[6]. Therefore, it is essential to understand the patient history to recommend the appropriate time and level of rehabilitation protocols that may be recommended after a PRP treatment. While some recommend rehabilitation along with any repair treatment[76] others suggest that resuming exercises a few days after any treatment may improve tendon's mechanical and biological parameters better than immediate loading or prolonged post-operative immobilization[77]. Currently, such recommendations in clinical trials are mostly subjective and are based on the practitioner. However, these recommendations should be based more on the patient and aim towards the "personalized medicine" approach.

Robustness of clinical trials

As mentioned earlier, in most current clinical trials investigating the efficacy of PRP treatment, the number of patients is relatively small (see above), which reflects the difficulties in recruiting a sufficient number of patients to participate in studies. A larger sample size in both control and treatment groups may likely increase the statistical power to detect the PRP treatment efficacy in clinical trials. These trials should take into consideration age, gender and other variables to minimize the inconsistent results obtained in PRP efficacy studies. Another variable that could affect PRP treatment efficacy is the measurement of PRP treatment outcome. Currently, various scoring systems are used as outcome measures; these include VAS (Visual Analog Scale), DASH (Disabilities of the Arm, Shoulder, and Hand), and VISA-A (Victorian Institute of Sport Assessment-Achilles) scale scores, which may be subjective because they are based on patients' assessment of pain intensity. Variations in these subjective scores can also be minimized by increasing the sample size in clinical trials and better yet,

using objective measures such as ultrasound imaging technology to reveal tendon structure before and after PRP treatment. Lastly, guidelines should be established so that the results obtained from different studies that administered PRP are uniform and easier to compare.

Changing the PRP treatment approach

The controversies surrounding the use of PRP in clinical trials are well-known. This issue can be best addressed by optimizing the application of PRP treatment in clinics. Currently, clinical trials employ a "one size-fits-all" approach where "any" PRP preparation is used at a "pre-determined dose" to treat all types of tendon injuries in patients regardless of patient age, gender or history. This means that PRP containing higher amounts of leukocytes or too many platelets may also be used to treat tendinopathy in older patients. All these variables can individually decrease the PRP treatment efficacy and therefore should be avoided. Thus, a "tailored" approach is necessary. For example, PRP efficacy may be higher when leukocyte free PRP is used to treat younger patients particularly athletes who may have large number of "good-quality" TSCs that may proliferate at a higher rate and differentiate into tenocytes. This approach may also be effective when treating early stage tendinopathy, which is characterized mostly by inflammation and pain because PRP is known to have anti-inflammatory effects[41]. However, PRP may not effectively treat advanced stage tendinopathy, which is characterized by degenerative changes such as lipid deposition, proteoglycan accumulation and calcification in tendon lesions[8]. Besides, the number of TSCs in these degenerate tendons is also small and will not allow effective PRP treatment; i.e. PRP can promote the proliferation of only the few existing TSCs, which may not be sufficient for an effective healing and repair. Therefore, it is recommended that degenerate tissues be removed by wound debridement prior to PRP treatment; this procedure will eliminate the non-tenogenic environment in advanced stage tendinopathic tendon and allow TSCs to proliferate at a higher rate[17]. It should be noted that when PRP is used to treat injured tendon-bone interface, a fibrocartilage zone which is more complex than tendon alone[27], additional "materials," like a bio-compound, together with PRP may be required to improve treatment outcome (Fig. 4). Finally, selective release of pro- or anti-angiogenic components in PRP using specific activators may

PRP alone is not sufficient to promote the formation of fibrocartilage zone in the tendon-bone interface

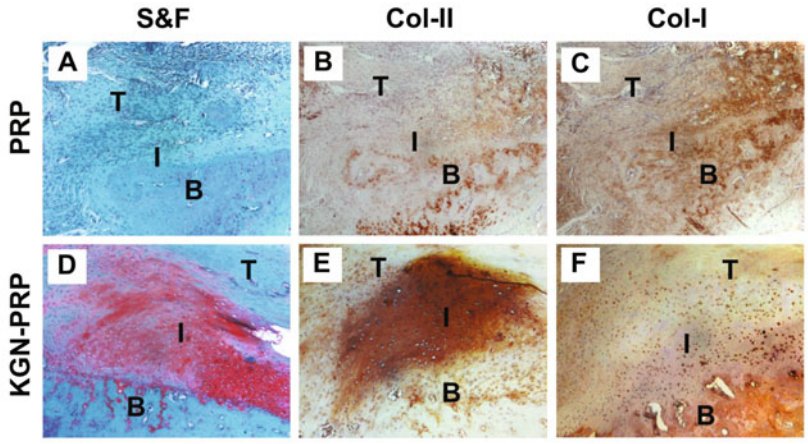

FIG. 4
Injection of PRP into the tendon-bone tunnel interface in a mouse model does not result in the formation of the fibrocartilage zone as evidenced by the lack of proteoglycan (A) and collagen type II (Col-II, B). In contrast, injection of KGN along with PRP induces robust formation of proteoglycan (pink, D), and abundant collagen type II (Col-II, brown, E). In both PRP and KGN+PRP groups, moderate amounts of collagen type I (Col-I, C, F) are formed. T - tendon; I – tendon-bone interface; B – bone.

For more detailed description of the results, see the original paper [27].

improve healing outcomes. All these converge on one point; i.e. if not monitored carefully, variations can occur easily during PRP treatment in clinical trials and may contribute to the reported inconsistent results.

10. CONCLUSION

PRP is an alternative option to traditional treatments of tendinopathy. PRP has a number of advantages; it is relatively safe and easy to use in clinics to treat tendinopathy. Findings from basic science studies have been consistent in demonstrating the beneficial effects of PRP including increased tendon cell proliferation, increased expression of anabolic genes and proteins, and reduced tendon inflammation among others. However, the efficacy of PRP in clinical trials is still controversial most likely driven by inconsistent protocols that use a "one-size-fits-all" approach. To obtain consistent results it is essential to use an individualized approach and optimize the variables related to PRP preparation and patients. Such efforts may improve the efficacy of PRP for the treatment of tendon injuries and may effectively address the controversies on PRP treatment efficacy in clinics.

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