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Perspectives on Improving the Efficacy of PRP Treatment for Tendinopathy

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Introduction

Platelet-rich plasma (PRP), popularly called PRP, is almost like a "house-hold" name these days because of its wide recognition by physicians and patients due to its prevalent use. The popularity of PRP is due to its efficacy in treating chronic tendon injury (or tendinopathy) in some clinical studies. It is particularly preferred by elite athletes because PRP injections were reported to accelerate healing and enable quicker return to sport activities [1,2]. However, there is no doubt that the popularity of PRP usage is also driven by financial gain. Typically, PRP injections are performed in out-patient clinics in a relatively short time period and a decrease in tendon pain and improvement in tendon function have been reported in patients with chronic plantar fasciitis [3], elbow tendinopathy [4-8], chronic Achilles tendinopathy [9-11] and patellar tendinopathy [12,13]. It has been estimated that more than 86,000 athletes receive PRP treatment every year in the United States and Europe [14]. In fact, the market value of PRP is estimated to grow to $126 million this year [15]. Despite such reports on the high efficacy of PRP treatment on tendon injuries, not all PRP treatments in clinics have been reported to be successful. For example, in some studies, PRP treatment did not improve tendon function and associated pain in patients with Achilles tendinopathy [16-18]. These conflicting clinical trial findings have raised skepticism in the use of PRP for the treatment of tendinopathy. However, despite critics opposing its use there is no decline in the use of PRP to treat tendon injuries in clinics, likely because PRP is autologous and has not been reported to harm the body by inducing immunogenic reactions, hyperplasia, carcinogenesis or tumor growth [19]. It is indeed made from a persons' own blood by one or two rounds of centrifugation processes such that it contains highly concentrated platelets in a small volume of plasma [20]. Therefore, the reported benefits of PRP and the lack of side effects thereof continue to fuel its use in clinics to treat tendon injuries. In this commentary, we attempt to discuss the scientific basis for the use of PRP to treat tendon injuries in clinics through a close analysis of the existing scientific data. We also provide suggestions to improve the PRP efficacy in clinics.

Growth Factors in PRP

A critical question to be answered about the use of PRP treatment in clinics is 'what confers the healing effects on PRP?' The consensus answer is *growth factors* (GFs), which both supporters and critics of PRP agree on. As the name suggests, PRP is the plasma portion of the blood containing platelets in high concentrations [21]. Platelets are the natural healing cells recruited to the wound area soon after a tissue injury; in addition to containing antibacterial and fungicidal proteins that prevent infections, platelets are reservoirs of numerous GFs such as 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), fibroblastic growth factor (FGF) and hepatocyte growth factor (HGF) [22-24]. GFs are in fact, the effectors that enhance the healing of tendon injuries by PRP due to their specialized functions [25,26]. For example, animal tendons treated with IGF-I [27], PDGF [28,29] or FGF [30] showed increased cell proliferation and collagen production. TGF-β also stimulated cell migration and proliferation in rat Achilles tendons [31] and increased collagen type I synthesis in tendon cells *in vitro* [32,33]. Further, our studies showed that HGF exerts anti-inflammatory effects on tendon cells *in vitro* and injured tendons *in vivo* [34]. Besides the benefits of GFs, PRP also contributes to tendon healing by forming a fibrin matrix when activated thus providing a conductive bio-scaffold for cell migration and new matrix formation [35,36]. Lastly, we can presume that the presence of physiological proportions of GFs in PRP may better benefit tendon healing than administering arbitrary proportions of GFs individually or in combinations [37]. Thus, the presence of GFs, fibrin matrix and "physiological" composition of GFs in PRP preparations may effectively drive the healing process of injured tendons after PRP treatment.

Recent studies on the PRP-induced cellular effects revealed that PRP exerts its effects on tendon stem/progenitor cells (TSCs), which are tendon specific adult stem cells discovered in the tendons of humans, rabbits, rats and mice [38-41]. *In vitro* treatment of TSCs with PRP in the form of platelet-rich clot releasate (PRCR) induced the differentiation of TSCs into active tenocytes, which are the dominant resident cells in tendons. These active tenocytes produced abundant collagen [25,42]. Similar effects were observed after the *in vivo* treatment of rat Achilles tendons with PRP and TSCs together [43]. From these studies, it may be inferred that PRP is safe to use for treating tendon injuries because PRCR, mainly containing releasates (GFs, cytokines, etc.) from activated platelets, did not induce non-tenocyte differentiation of TSCs [25,42], which may lead to the formation of non-tendinous tissues such as fatty-, cartilage-,

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or bone-like tissues, thus compromising tendon structure and therefore causing more damage to the treated tendons. Based on these previous findings, it can be concluded that there are basic science data that support the use of PRP to treat tissue injuries in general and tendinopathy in particular because GFs and possibly other factors in PRP can reduce tendon inflammation and hence pain, and enhance tendon wound healing by exerting their anti-inflammatory and anabolic effects on tendon cells [34].

PRP Treatment Variations

Scientifically speaking, if PRP treatment for tendon injuries is reasonable, then why do clinical trials report many conflicting results? We believe that multiple factors normally practiced in clinical PRP treatments may critically influence the PRP treatment outcome. These include patient-related and PRP-related factors.

Patient-Related Factors

First is a list of variables related to patients; examples of these include variations in age, gender, recommendation or not of rehabilitation along with PRP treatment, disease history, treatment history, etc. Existing data suggest that age may be a critical factor in determining the efficacy of PRP treatment because aging affects stem cells in two ways; it decreases their number as well as reduces their quality. For example, the number of MSCs in human bone marrow [44] and the number of TSCs in mice [45] and rat tendons [40] declined with age. Similarly, stem cell qualities such as stemness, proliferation and differentiation capacities also decreased with age in humans and animals. Specifically, adipose-derived stem cells (ADSCs) [46], bone marrow stem cells (BMSCs) [46], TSCs [40,45] and ACL-derived cells [47] from young animals proliferated at a higher rate than those from older animals and, the "fitness" of human MSCs [44] reduced with age. Moreover, the fate of stem cells were also altered in TSCs with more cells from aging rats and mice differentiating into non-tenocyte lineages such as adipocytes, chondrocytes and osteocytes [40,45]. Aging not only diminishes TSC properties, but also has its effects on GFs; increased numbers of GF receptors were observed in young than older porcine ACL fibroblasts [48] suggesting that GF receptors could be more in young tendons than older tendons although evidence for this is warranted. In addition, PDGF concentrations were higher in P-PRG (pure-platelet rich gel) derived from young horses than older horses [49]. Thus, the increased number of GF receptors and higher amounts of GFs in young individuals may trigger a better cellular response to PRP treatment in young than older individuals. These findings shed some insights on why PRP treatment may be less effective or even ineffective in older patients who may have fewer numbers and "poor quality" of stem cells [20]. Therefore, the efficacy of PRP treatment in healing tendon wounds in aged patients is expected to be low. Also, current clinical trials mostly include patients of various ages ranging from 18 - 70 years old [17,50-52] that may likely skew the results due to variable PRP treatment outcomes because PRP treatment in older individuals may be poor or non-effective. Therefore, clinical trials should have age as an inclusion criterion for PRP treatment; separating young and old cohorts may improve the PRP treatment outcome: a smaller number of young patients is needed to demonstrate the efficacy of PRP treatment for tendinopathy, whereas a larger number of older patients is required for the same efficacy simply because of large variations in treatment effects in older patient populations. The age may be an important factor that causes inconsistent results in clinical trials.

Gender could be another main determinant of PRP efficacy because of known gender differences in the viscoelastic properties of tendons [53], incidences of Achilles tendon ruptures [54,55] and wound healing [56]. These reports indicate that sex may influence the PRP treatment outcomes due to inherent differences in the biological properties of tendons between males and females that may impact their response to PRP treatment differently. However, in most clinical trials, males and females are grouped together with significantly larger number of males than females, which may confound the PRP treatment efficacy. In a recent study, the levels of all GFs were reported to be higher in human females than males with significant differences in the levels of EGF, HGF, IGF-I and PDGF [57]. Similarly, the concentration of PDGF in P-PRG derived from female horses was significantly higher than from male horses [49]. Lastly, the PRP treatment in aged or post-menopausal females should be given particular attention because reduced estrogen levels have been linked to decreased tensile strength [58] and diminished wound healing response of dermal fibroblasts [59,60], and exogenous estrogen has been shown to improve tendon morphology and biomechanical properties post-menopause [61], and regulate VEGF [62] and IGF-I expression [63]. To effectively evaluate the issue of gender in PRP treatment, clinical studies should separate patients based on gender prior to treatment. Moreover, to demonstrate the efficacy of PRP treatment on tendon injuries, male and female patients should be separated or only one sex should be included in clinical trials to reduce variations due to gender and hence increase statistical power of a clinical trial. On the other hand, if both male and female subjects are included in a clinical trial for PRP treatment, a much larger sample size (the number of patients in both control and treatment groups) must be adopted to increase statistical power to detect the effects of PRP treatment.

Another patient-related factor that should be addressed is whether or not to recommend rehabilitation with PRP treatment. A study investigating the effects of PRP with or without mechanical loading on rat Achilles tendons found that nullifying loading with Botox injections negated the PRP treatment effects while in normal tendons (not treated with Botox) both PRP and mechanical loads independently increased tendon repair without synergistic effects [64]. A randomized control trial (RCT) also studied the effect of eccentric exercise after PRP treatment on patients with lateral elbow epicondylitis [8]. This study showed that the VAS scores were improved in patients in the exercise group in comparison with the control group that remained sedentary [8]. In general, most studies suggest that strengthening exercises may benefit tendon structure and function although the timing of applying rehabilitation seems questionable with some suggesting resuming activity immediately along with any repair treatment [65] and others arguing that a slightly delayed loading may improve mechanical and biological parameters to a greater extent than immediate loading or prolonged post-operative immobilization [66,67].

Another recent addition to the patient-related factors is the likely presence of single nucleotide polymorphism (SNPs) in the genes transcribing GFs and other PRP-related factors [68]. Although direct evidence for the presence of SNPs in GFs and how they affect PRP treatment efficacy has not been established yet, the IGF2 GC genotype was found to reduce the degree of muscle injuries in elite soccer players when compared to those with IGF2 CC or GG genotypes [69]. Similarly, a SNP in elastin gene (AA genotype) was found to predispose patients to severe muscle injuries and prolong recovery times compared to the AG or GG genotypes [70]. It remains to be seen whether such SNP occurrences influence the PRP efficacy treatment in clinical trials.

Considering the above discussions together, we suggest that clinical trials of PRP treatment on tendon injuries should be large, randomized and selective in patient enrollment so that the statistical power of the PRP treatment outcomes in trials can be enhanced. On the other hand, more basic science studies are also needed to obtain a comprehensive understanding of the age, gender and rehabilitation effects on the PRP treatment efficacy. Other factors such as genetics, disease history and prior treatment history after PRP treatment should be considered as variables in clinical trials. Finally, in clinical trials of PRP treatment, appropriate selection of controls is critical in assessing the efficacy of PRP treatment. These controls may include needle penetration and saline injection. We believe that platelet-poor plasma (PPP) injection may be the best control because it includes needle penetration, and plasma contained in most PRP preparations [34].

PRP-Related Factors

The second major factor that may cause inconsistent results

in PRP treatment efficacy in clinics is related to PRP itself. This includes differences in the platelet concentration, PRP composition and activation method. In clinical studies, PRP is prepared using commercial kits that do not yield "one-type" of PRP with the same composition. The main difference in PRP preparations appears to be in the platelet concentrations. Indeed, manufacturers describe that in current commercial preparations platelet yield could increase by 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 up to 10-fold (GenesisCS) higher than in whole blood depending on the kit used. While the rationale to use PRP is to deliver concentrated GF containing platelets at the site of injury, 'more' does not always translate to 'better'. No improvement in tendon function (ATRS score) was observed when PRP containing 10-fold more platelets than in whole blood was used to treat acute Achilles tendon ruptures in patients [18]. Therefore, most clinical and basic science studies only use PRP with platelet concentrations 3-5-fold higher than in whole blood due to apparent beneficial effects; decreased pain, increased tendon cell proliferation, improved collagen synthesis and organization, etc. [42,71-73].

Besides platelet concentration, PRP composition may differ. Specifically, leukocyte levels in PRP preparations may vary and can affect PRP treatment outcomes. While leukocytes promote chemotaxis, cell proliferation and differentiation [74] they also release inflammatory cytokines such as interleukin-1 β (IL-1β), tumor necrosis factor-α (TNF-α) and reactive oxygen species that can harm the already injured tissues [75]. Indeed, leukocyte containing PRP (L-PRP) inflicted catabolic effects on human chondrocytes [76] and rabbit TSCs [73]. More importantly, pure PRP (P-PRP) or leukocyte free PRP promoted anabolic effects by increasing collagen II and aggrecan expression in chondrocytes [76] and upregulating alpha-smooth muscle actin (α-SMA), collagen types I and III in rabbit TSCs [73]. These and other studies suggest that to promote tendon healing it may be beneficial to use PRP with low concentrations of leukocytes.

Although the platelet and leukocyte concentrations in PRP can be easily optimized, thus far, a standard PRP composition to treat tendon injuries has not been worked out. The lack of guidelines and inconsistencies in the PRP preparations made from kits skew the PRP efficacy reported in clinical trials. A comparison between MTF Cascade, Arteriocyte Magellan and Biomet GPS III PRP preparation systems using the same blood sample showed no significant differences in platelet concentration but huge variations in the concentrations of leukocytes, PDGF and VEGF [77]. Similarly, the platelet concentration was similar in aphaeresis-derived platelets, Buffy coat-derived platelets and tube method-derived platelets. However, the leukocyte concentration differed significantly between the samples [78]. If any of these leukocyte containing PRP preparations are used to treat tendon injuries, the end result would be the induction of catabolic effects that may skew PRP treatment efficacy.

Another less frequently discussed PRP-related factor is platelet activation that releases the GFs stored in alpha granules [78]. Traditionally, PRP is activated using an external source such as thrombin, calcium chloride or collagen [11,64,79]; however, some authors inject non-activated PRP because platelets become activated *in vivo* when in contact with collagen [8,37].

Due to these variations in PRP-related factors, some authors have suggested a universal PRP labeling system to be followed with description of PRP components. Some examples of classification systems are the use of PAW, which indicates the Platelet concentration, Activation method and presence of White blood cells or leukocytes [80] or DEPA, which indicates the Dose of platelets, Efficiency of production, Purity of the PRP and Activation of PRP [81]. While the various technologies and in-house preparation methods provide flexibility to choose a kit/method for clinical use, careful consideration should be given to the resulting PRP preparation for the intended treatments. For instance, tendon injuries in the early phase of healing are mainly inflammatory [82-84], and may be treated with P-PRP because of its anabolic and anti-inflammatory effects. In the early phase, tendons are not degenerative and P-PRP is likely to induce TSCs to proliferate and differentiate into tenocytes and as a result, enhance tendon healing. However, advanced stage tendinopathy may not be treatable with P-PRP because this stage is characterized by degenerative conditions with extensive formation of lipids, proteoglycan accumulation, and calcification in tendon lesions, either alone or in combination [85]. With only few TSCs remaining in the degenerative tendons, P-PRP may not be able to enhance sufficient TSC proliferation to repopulate the degenerated tendon, promote collagen production or reverse the already differentiated non-tenocyte cells; therefore, an effective repair of advanced stage tendon injury by P-PRP is unlikely. However, surgical removal of tendon lesions (wound debridement) has been advocated in clinics to induce healing in a non-tenogenic environment in advanced stage tendon injuries [35,86]. This debridement procedure may be followed by L-PRP treatment, which due to the presence of leukocytes, may kick-off a healing process in injured tendons. Finally, platelets in PRP are known to contain pro-angiogenic (e.g. VEGF) or anti-angiogenic (e.g. endostatin) factors [87] that may have differential effects on the healing of tendon injuries. These factors are also selectively released after platelet activation by specific protease activated receptors, PAR1 or PAR4. Therefore, future studies should investigate the effect of selective platelet activation and its influence on the healing of tendon injuries.

Conclusion

Considering the above discussion, it seems reasonable to use PRP to treat tendon injuries effectively because it can reduce tendon inflammation and hence improve tendon function and accelerate tendon healing, mainly due to the abundant GFs released from the platelets in PRP once activated. It is apparent that the current "one size-fits-all" approach to treat "any" tendon injury using "any" PRP preparation would almost certainly lead to contradicting results or would diminish the efficacy of PRP treatment. Thus, instead of the "one-size-fits-all" approach, a "tailored" approach with considerations on the patients' age, gender, disease history, rehabilitation, etc., and an appropriate type of PRP, must be applied to treat tendon injuries with PRP so that the maximum efficacy of PRP treatment can be achieved in clinics.

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