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ADVANCES WITH PLATELET RICH PLASMA THERAPIES FOR TENDON REGENERATION

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ABSTRACT

Introduction: PRPs can be used in the management of tendinopathy if we improve our understanding of pathophysiology and achieve to integrate molecular knowledge about PRP participation in healing mechanisms.

Areas covered: We provide new insights into the pathophysiology of tendinopathy, PRP therapies, and the potential links between both. We discuss the place of PRP in promoting tendon repair within what is currently understood of the role of PRP molecules in promoting tendon regeneration. We also highlight the many opportunities

for further exploration and identification of strategically designed treatments providing meaningful clinical benefit.

Expert opinion: Development of PRP treatments is challenging because the typical group of patients with tendinopathy does not exist, as it affects multiple segments of the population. Moreover, the pathophysiology and origin of pain are not elucidated yet. Although some degree of success has been achieved, PRP is not considered standard medical treatment, nor is it paid\reimbursed by insurance companies. However, the arguments for using PRP in tendinopathy are increasing, and its potential to rebalance inflammation merits further research. Moreover, PRP contains tendoinductive factors that can drive the fate of stem cells. Tailoring PRPs to the specific needs of the host tendon has not been possible to date, because unanswered questions remain about the characteristics of tendinopathy within the different stages of progression. PRP can be part of a tendinopathy management program, including load and pain management.

Key words

Cells, cytokines, growth factors, healing, platelet-rich plasma, tendinopathy

Highlight box

- The foundation of PRP application is to modify the molecular milieu by providing supraphysiological concentrations of platelets (and optionally leukocytes) at the injured/pathological tissues mimicking the initial stages of healing.
- However, the efficacy of PRP is controversial in tendinopathies, given the contrasting results from well performed randomized controlled trials.

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- When extending current knowledge about PRP efficacy, it is important to consider the diversity of PRP formulations and provide clinical results with the full description of the PRP used and the protocol for application.
- The simplest way to customize PRP for the specific application consists of selecting among clinically available formulations, the timing of application, as well as the number of doses. These essentials of PRP treatment have not yet been optimized.
- Another logical step forward is to produce combinatory treatments by identifying what really matters to associate PRP with selected molecular inhibitors or enhancers for each clinical application. PRP has been combined with different cell phenotypes to treat tendon conditions.
- PRP is not a monotherapy; it should be used as part of a tendinopathy management program including load and pain management.

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1. INTRODUCTION

Tendinopathies are musculoskeletal conditions often induced by cumulative tendon microinjuries at sites of strain. Likewise, tendon conditions can result from excessive overload or trauma provoking acute macroscopic tendon tears. Clinically, tendinopathies are characterized by focal tendon soreness, lessened strength, pain upon activities and progressive reduction in function [1]. Habitually, tendinopathy is irreversible, as tendons do not heal because of their limited biological resources for repair [2]. Currently, there are no satisfactory treatments for tendon conditions, and they remain an important unmet medical need [3].

The burden of tendinopathy affects differentially diverse populations, including athletes, workers and ageing people, with different anatomic vulnerability and economic burden [4]. The prevalence of pathology and affected tendons vary according to biological background, mechanical requirements of the muscle-tendon unit, and endogenous risk factors [5]. On the field of sports, the location of tendon injuries is often sport-specific, interfering extensively with practice, training and competition. For example, Achilles tendinopathy has 18% prevalence in recreational and competitive runners [6], whereas patellar tendinopathy is common among jumping athletes. On the other hand, elbow lesions (medial or lateral epicondylopathy) present frequently in the working population performing repetitive movements, and have 7% prevalence in manual workers versus 1-3% in normal population [8]. Likewise, the incidence of rotator cuff pathology is around 30% in the general population, and the prevalence of rotator cuff tears increases with age [9-10]. Patients' age also increases the probability of having other concomitant risk factors for tendinopathy, including hormonal imbalances, obesity, diabetes and other metabolic diseases [5, 11].

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Over the past decades PRPs have emerged as a biological treatment to repair or regenerate impaired or non-functional tissue in different medical areas. From the beginning, they were adopted by sports medicine physicians and orthopedists with high expectations of PRP performance, aiming to accelerate return to play [12]. Media coverage of anecdotal outcomes in elite athletes and other celebrities raised fascination in the public [13], and enhanced demand of PRP treatments. However, a robust scientific understanding of how PRP works and clinical evidence supporting specific indications take time and resources to develop and are not available yet.

The molecular complexity of PRPs and its interactions with the different presentations of the host tissue constitute the core of PRP research. PRPs can serve to manipulate and enhance healing if we integrate molecular knowledge about PRP and its participation in healing mechanisms. An additional layer of complexity, very inciting for the researcher to challenge, is characterizing the host tissue conditions, namely stage of the disease and altered mechanisms in the pathological tendon with which PRP has to interact.

Here we provide insights into the current understanding of PRP therapies, as well as the recent advances in our knowledge of the pathophysiology of tendinopathy and the potential links between the two. To provide a framework for this link according to the most recent literature, we performed a comprehensive search in PubMed and MEDLINE using “tendon” and “platelet-rich plasma” as MeSH terms, and selected relevant articles published in the last five years. We also highlight the many opportunities that exist for further exploration and identification of strategically designed treatments associated with meaningful clinical benefit.

2. NEW INSIGHTS INTO THE PATHOGENESIS OF TENDINOPATHY: IMPLICATIONS FOR PRP TREATMENT

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Theories about the pathophysiology of tendinopathy are largely unproven, and may diverge depending on tendon type because of unique mechanical requirements and anatomical specificities. Broadly speaking, they can be reduced to two hypotheses, not mutually exclusive: first the "continuum model" [14], and second the "failed healing theory" [15-16]. The former consists on initial reactive tendinopathy, followed by tendon disrepair and degenerative tendinopathy with no evidence of inflammation throughout the course [14]. Tendinopathic changes are typically progressive and silent, hampering research in early disease stages, as patients are asymptomatic until they reach a threshold [17].

On the other hand, according to the "failed healing" theory, [15-16] stalled healing mechanisms generate an innervated angiofibroblastic tissue, which is often painful and with reduced mechanical properties. Different interconnected biological processes, including inflammation, neuronal proliferation, imbalanced anabolism/catabolism and dysregulated apoptosis, lie beneath the pathology.

2.1 Inflammation

Recent research has provided crucial information emphasizing the relevance of inflammation, as a signal mediated response to both injury and loading, and its failure to resolve in tendinopathy [18-19]. Various cell types, through their crosstalk with positive and negative feedback loops, shape the main features of inflammation. Upon tendon injury, three cell sets, including infiltrating immune cells (i.e. monocytes/macrophages, lymphocytes), resident immune cells (mast cells [20], and tissue macrophages) along with stromal local cells (tenocytes and precursor cells) crosstalk and synchronize their activities to achieve healing or failed healing [21]. Converging themes in the pathophysiology are endogenous and exogenous risk factors that confer vulnerability to develop tendinopathy **(Figure 1).**

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Loading modifies these interactions, thereby producing a network linking inflammation, loading and healing. Interestingly, a similar expression of inflammatory molecules [22] and ECM related genes (COL1, COL3 and TGF-β1) [23] were achieved upon tissue stress, no matter the triggering agent: physical aggression through needling or mechanical loading, indicating a convergence of these pathways.

Inflammation is a temporal and spatial concept, involving molecular and cell adaptions to stress. Contradicting the historical dogma of lack of inflammation in tendinopathy, a recent review of all data in the literature revealed increased numbers of macrophages and mast cells in tendinopathic tissue [24].

For effective resolution of inflammation to occur, differentiation of macrophages is required. In fact, macrophages adopt a continuum spectrum of different phenotypes, from inflammatory, that is M1 phenotype expressing IL-1b and other pro-inflammatory molecules, to healing phenotype, M2, synthesizing growth factors, cytokines and specialized pro-resolving mediators (maresins) [25]. Balanced intensities and timing of macrophage transitions are key drivers of tendon regeneration and have become the focus of new therapeutic approaches [26].

Animal models reinforce the association between inflammation and healing and also emphasize the importance of loading and the network thereby produced. This was confirmed in an Achilles tendon transection model, in which the temporal pattern of immune cell infiltration of rats receiving botox into calf muscle was compared with cage free moving rats. Loading prolonged the M1 phase (delayed the switch of M1-M2 macrophages assessed as CCR7/CD206 ratio) with more Treg cells (Treg/Thelper ratio), and made the tendon regenerate bigger [27].

Although M2 polarization is needed for the resolution of inflammation, excessive M2 activity can result in a fibrotic tendon. Consistent with this, obese mice with type II

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diabetes showed fibrotic tendon healing from excessive M2 activity [28]. As in other biological processes, a delicate balance between positive and negative feedback loops drives the resolution of inflammation and the quality of tendon repair.

Stromal tendon cells are not just passive players. They mediate inflammation by polarizing into pro-inflammatory states, synthesizing IL-6, IL-8, GRO-a, RANTES and MCP-1, after exposure to IL-1β [29]. Interaction with IL-1β also enhanced MMP/TIMP ratio. Furthermore, exposing normal tendon explants to IL-1β showed degradative processes of ECM cleavage and release of COMP analogous to tendinopathy [30]. In supraspinatus tendinopathy, inflammation was present in stromal cells with a different molecular signature, depending on the stage of severity. Data revealed activation of IFN-ɣ and NF-kB in the initial stages and STAT6 and glucocorticoid pathways in advanced stages [30].

Compromised cell survival [31] from crucial changes in ECM composition [32] is hypothesized in tendinopathy. On cell death, intracellular molecules named "alarmins" are released to the extracellular space. They act as DAMPs signaling tendon stress through TLR activation in immune cells [33] (reviewed in Millar NL 2013). Immunohistochemistry has revealed increased alarmin S100A9 and HIF-1α in painful rotator cuffs compared to non-painful diseased tendons, whereas a different alarmin, HMGB1, was increased in non-painful tendons. In addition, IL-33 was reduced in diseased tendons compared to controls [34]. IL-33 is also involved in collagen synthesis, so it provides a link between inflammation and ECM synthesis. Further experiments using explant cultures exposed to IL-1β in supraspinatus tendinopathy revealed different gene and protein expression pattern (IL-6 and IL-8) than healthy controls. Immunostaining and flow cytometry also confirmed differences in podoplanin, VCAM-1 and CD106 [35]. Overall, these experimental data support

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evolving inflammation with different molecular patterns according to disease stages and pain symptoms [36-37].

As proposed by a recent paradigm, resolving rather than suppressing inflammation might be necessary for tendon healing [18]. In this context, it is necessary to identify molecular checkpoints that limit inflammation and enhance ECM synthesis. In this regard, IL-33, IL-17A and IL-6 [38-39] were mechanically regulated and identified as molecular links between inflammation and collagen synthesis. Building on the overall data above we can envisage future research on molecular panels of biomarkers to assess the stage of disease and develop personalized biological treatments.

2.2 Fibrosis

Tendon fibrosis, a feature of tendinopathy, impairs normal ECM tension because of excessive deposition of collagen 1. As tendons are mechanosensitive, these changes in matrix stiffness compromise the maintenance of tendon fibroblast phenotype inducing their differentiation into myofibroblasts. Morita et al. [40] reviewed human and animal studies to explore the potential involvement of fibrotic factors, including TGFβ, BMPs and CTGF in tendon conditions. Their expression was altered during healing in both acute and overuse injuries [40].

2.3 Neuronal dysregulation

Importantly, neuronal dysregulation is a feature of tendinopathy and is related to pain perception. Thus studies comparing tissues from painful versus pain-free patients can help in clarifying pain features in tendinopathy and the lack of correlation with tissue pathologic changes. According to published data reviewed by Dean et al. [41] there were clear changes in the peripheral neuronal phenotype with important involvement of glutamatergic transmission, through both metabotropic and ionotropic glutamate

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receptors [42]. In addition, autonomic, and sensory pathways contribute to pain with spatial and temporal differences [43].

Different anatomical locations can display different patterns of immune response and distinct neural signaling. For example, nerve fascicles containing sensory afferents are present in the peritendinous tissue of painful epicondylopathy [44]. Upper limb tendinopathy was associated with central nervous system sensitization whereas patellar and Achilles tendinopathies showed a peripheral pain state [45].

3. PLASMA THERAPIES IN TENDINOPATHIES

Several hypotheses about PRP interfering with the pathogenesis and perpetuation of tendinopathy have been explored as explained below. Currently, there is no irrefutable evidence that PRP regenerates tendons structurally [46-47], even if clinical symptoms lessen in most controlled studies [48]. But, in a therapeutic continuum, partial healing or catabasis with remission of symptoms are plausible at this stage of development of PRP technology. Certainly, as we discuss below, PRP formulations have to be tailored to specific conditions of the host tissue to obtain meaningful clinical efficacy. The use of combination products (cells + PRP) is also plausible in advanced conditions [49-51].

3.1 Mechanisms of action

According to the failed healing hypothesis, the ideal tendinopathy treatment should modify the pro-inflammatory response and promote resolution actively, encouraging robust and rapid matrix repair. Inducing an acute burst of inflammation in chronic tendinopathies may finish up initiating its resolution and subsequent healing.

PRPs can modulate inflammation and healing through different modes of action, not mutually exclusive. First, activated platelets can establish direct platelet-immune cell interactions. In doing so, they can reduce the proliferation of T lymphocytes and their

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differentiation towards T-helper type 17 lineage [52]. Using direct cell-binding mechanisms, platelets can also modulate the actions of macrophages [53] and their polarization state, which is associated with persistence or resolution of inflammation. Direct platelet-cell interactions can be favored by using activated platelets re-suspended in buffer without plasma.

Second, upon activation of the coagulation cascade, PRPs release a large pool of extracellular ligands (the so-called secretome) that exert their actions via agonist properties at cognate receptors. In doing so, they regulate multiple cell functions, including inflammation, proliferation, differentiation, apoptosis and matrix anabolism. All these mechanisms can be activated because all this pool of extracellular ligands signal through different receptors associated with diverse signaling pathways. These interactions trigger diverse expression patterns on various cell types depending on the constitutive and inducible receptor expression. In addition, but less explored, microparticles and exosomes (nano and microvesicles) are involved in intercellular communication and can modulate tissue responses [54-55].

Lastly, indirectly, PRPs can modify the biological status of pathological tissue by occupying the physical space, thereby altering the cytokine profile and removing signals of inflammation or ECM catabolism. This steric washout mechanism is favored by using high volumes and might be assimilated to saline injections ("placebo"). It does not exclude former described actions.

According to these three mechanisms of action, spatial delivery of the product matters as cell signatures in the proper avascular tendon differ from those in the enthesis and surrounding vascularized structures, including endotenon, paratenon, tendon sheath or bursa [56-57]. Furthermore, the design of specific treatments for the enthesis could be anticipated. When PRP was combined with kartogenin, fibrocartilage was formed in

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enthesis lesions produced with a biopsy punch [58]. This combination treatment, PRP plus kartogenin, also enhanced tendon graft integration within the bone tunnel during ACL reconstruction in a rat model [59].

3.2 Main cellular targets of PRP

In the last few years, advances with PRP for tendon regeneration were focused first on immune-modulatory actions, and second on stromal cell biology (tenocytes and precursors) in laboratory controlled experiments. In vivo, both actions occur in parallel as PRP modulates the crosstalk between the different cell phenotypes.

3.2.1 PRP and innate immune cells

PRP is immunomodulatory acting on both the infiltrative and local immune cells, i.e. resident macrophages and resident mast cells. Catecholamines, histamine and other metabolites in PRP induce vascular permeability and favor transmigration across the endothelium of various leukocyte subsets. PRP application produces gradients of chemoattractants for neutrophils by means of β-thromboglobulin (also known as neutrophil activating peptide-2, (NAP-2)), CXCL1/GRO, ENA78/ CXCL5, and IL-8 among others. In addition, infiltration of monocytes is driven by MCP-1 and RANTES gradients.

Other abundant molecules within PRP can modulate the polarization of macrophages. For example, PF-4, representing 25% of the α-granule content has been involved in macrophage polarization [60]. However, genome wide expression profiling data in rats did not show any effect on macrophage polarization when PRP was compared to the PPP [61]. Apparently, no clear single definite pathway is strongly targeted with PRP, but according to genome wide expression arrays modulated pathways included NF-kB and TNF-α signaling when compared to PPP. This is part of a larger program, as a

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decreased expression of ECM genes and enhanced autophagy related genes and ROS were also identified) [61].

3.2.2 PRP and stromal tendon cells

At the beginning, the common paradigm of PRP healing actions was based on the proliferative potential of growth factors, crucial for achieving a critical number of local stromal cells. PRP increases proliferation of tendon cells by modulating Stat3 and p27 to up-regulate expression of cyclins and cyclin-dependent kinases [62].

However, PRP research should not be restricted to growth factors but broaden to take into account the biological actions of small molecules released from dense granules. Furthermore, the crucial signaling actions of cytokines and chemokines form part of a larger regeneration program.

Stem cells have great importance in PRP therapies, as they contribute to healing or non-healing. Current data indicated that there might be different sources of tendon stem cells with multilineage potential: vascular and non-vascular. The former can be found in epitenon and endotenon, harbored by the wall of capillaries and blood vessels [63], and the latter in the proper tendon [64].

PRP can enhance mobilization of stem cells from other sources to the location of the injury [65]. Actually, platelets provide initial cues, such as PDGF-B, bFGF and CXCL5, for the homing of circulating precursor cells to the injury [66]. PF-4 works in cooperation with PDGF and CXCL7 to activate fibroblasts' migration [67]. As a tendon is connected to muscle, stem and progenitor cells of skeletal muscle might also be a possible source of stem cells for tendon repair after injury.

Not only migration, but the fate of these endogenous stem cells can be influenced by the cytokines contained in PRP. Recent work has shown that platelet-rich releasates (that is the soluble fraction obtained after the coagulation of PRP) promoted tenogenic

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differentiation of progenitor cells [68-69] and leukodepleted PRP was safer than leukocyte-rich PRP (L-PRP) [70]. Animal experiments [70-71] corroborated these hypotheses as activation of the niche with PRP promoted regeneration of rat Achilles tendon in two models: acute rupture in rats [71] and collagenase induced tendinopathy [65].

Most of the signaling molecules from α-granules are active in the picogram to nanogram dose-range. But platelet factor-4 (PF-4) is in the microgram range [72].

Migration, proliferation and adequate differentiation underlie regeneration. Data from individual actions of growth factors (isolated from the full molecular pool of PRP) can serve to formulate hypotheses, but cannot be extrapolated. In this sense, in vitro and in vivo data [73] revealed activation of the tendon niche with CTGF (present in PRP), in particular CD146+ TSCs. These cells underwent robust proliferation and differentiated into tenocytes after 7 days, leading to collagen organization and tendon regeneration.

Importantly, the activities of platelet secretome and plasma circulating elements are regulated at multiple levels. In particular TGF-β, IGF-I and HGF are inactive while circulating and need proteolytic activation to participate in healing [74-75]. Recent data indicate that tissue injury activates a pro-HGFA enzyme, which in turn renders HGF active. This is an important mechanism: HGF interaction with the local niche enhances tendon regeneration [76].

3.3 PRP and apoptosis

Platelets control the balance from apoptosis towards cell survival by secreting mediators with anti- and pro-apoptotic functions [77]. This is relevant for the treatment of tendon conditions as excessive apoptosis was identified [31]. Platelets' microparticles can enhance survival through phosphorylation and activation of Akt, which inactivates the pro-apoptotic BCL-2 family member BAD [55]. Antiapoptotic effects of PRP can also

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be attributed to PRP secretome, including HGF, SDF-1α, serotonin, ADP, and sphingosine-1-phosphate IGF-1, IGFBP1, IGFBP2, BDNF, TIMP-1, PROC, INHBA, TAC1 [78]. On the other hand, platelets release TNF-α related ligands, which are pro-apoptotic, including CD95 (FAS-L), CD154 (CD40L), Apo2-L (TRAIL), Apo3-L (TWEAK), and LIGHT [76]. Mostly, PRP can augment the molecular milieu and decisively define the delicate balance between pro- and anti-survival molecules.

Furthermore, in the actual context of tendon management with anesthetics [79] and corticosteroids [80] (reviewed in Abate 2017), local cytotoxicity is acknowledged and could be potentially counteracted by PRP. Whether PRP affords protection against the deleterious effects of lidocaine and other commonly used aminoamide local anesthetics (such as ropivacaine, mepivacaine or bupivacaine) and corticosteroids is controversial. Inhibition of apoptotic pathways (i.e. inactivation of pro-apoptotic proteins, regulation of caspases' activities) or activation of cell survival pathways has been explored in vitro but timing and doses strongly influence results, which are not conclusive.

3.4 Differences between plasma formulations

Different formulations, with differing biological effect in vitro, are obtained depending on the method of preparation or commercial protocol. The hypothesis that clinical outcomes were influenced by PRP formulation encouraged the development of classification systems according to constituents and their stoichiometry [81]. In experimental research, studies comparing leukodepleted PRP and L-PRP, showed that the presence of leukocytes and platelet count influenced inflammation and matrix turnover (MMPs, ADAMTs, TIMPs). In vitro 3D cultures showed that L-PRP and PRP are chemotactic for tendon cells from healthy and pathological tissues. PPP did not stimulate cell migration. On the other hand, L-PRP is more pro-inflammatory than pure PRP or PPP in vitro as assessed by gene expression and further confirmed with protein

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assessments (IL-6, IL-8, MCP-1, GRO-α, RANTES) [82]. Accordingly, L-PRP induced higher inflammation (enhanced TNF-α and IL-1β/IL-1ra ratio) than pure PRP in tendon explants [83] and also in tendinopathic cells [84]. Animal models of collagenase induced tendinopathy confirmed these data. Intra-tendon delivery of pure PRP was better than L-PRP as assessed by MRI, transmission electron microscopy and histology (analyzed under polarized light microscopy). Four weeks after treatment, L-PRP treated tendons displayed higher signal intensities on T2 mapping indicative of inflammatory edema [85]. In addition to being pro-inflammatory, L-PRP induced non-tenocyte differentiation of tendon stem cells [67-68]. PRP and PPP stimulated cells to produce ECM in more degree than L-PRP. The number of platelets [86] and the ratio platelets:leukocytes [87] influenced the synthesis of ECM, with higher platelet concentrations being detrimental for tissue anabolism.

Up to 2014, most clinical studies reported the use of L-PRP (in 97% of patients) [88], but later studies are testing the efficacy of pure PRP. Direct clinical comparisons between both products in tendinopathy are lacking.

Biological differences between these formulations can improve our treatments, but we have to establish specific indications. Furthermore, we have to consider the specific location of the injury (i.e. musculotendinous or enthesis), as muscle injuries can heal better with plasma without platelets (i.e. platelet poor plasma, PPP). The reason is that PPP promotes the differentiation of satellite cells into myoblasts instead of promoting proliferation, as occurs with PRP [89].

4. HOW EFFECTIVE IS PRP IN TREATING TENDINOPATHIES?

Although some degree of success has been achieved, PRP is not considered standard medical treatment nor is it paid by insurance companies because of the lack of strong evidence about is efficacy and non-demonstrated cost effectiveness. However, clinical

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research is evolving continuously, and data from new randomized clinical trials can help to overcome heterogeneity in meta-analyses. In general, pain relief is the main outcome measurement used when pooling data from different studies performed at different anatomical locations [48,88]. Other measures of outcome include patient self-reported scores. However, the variability of formulations, volumes, procedures for application, number of interventions and interval between them as well as the heterogeneity of outcome measurements and comparators, limit the applicability of these statistical results.

Fitzpatrick et al pooled data from 18 clinical studies, 17 of which had low or medium risk of bias involving all anatomical areas, performed a network meta-analysis and found that PRP is more efficient than other treatments. Moreover, they found that LR-PRP yielded better outcomes than leuco-depleted PRPs. However, these analyses of data are blurred because of the heterogeneity of the comparators. These results might fit with the hypothesis of unresolved inflammation and triggering a bout of inflammation with L-PRP thus activating subsequent healing mechanisms.

Reviews and meta-analyses focusing on particular anatomical areas are imperative, as lower and upper limb tendinopathies differ with different segments of the population being affected. Using the subgroup approach, patellar tendinopathy, and epicondylitis have been reviewed. The problem when performing subgroups is power reduction. For example, a very recent review and meta-analysis [90] of PRP intervention in patellar tendinopathy found only two eligible randomized controlled studies [91-92], involving one and two injections respectively for recalcitrant patients. Control groups differed (ESWT and dry needling). When pooling all the results, PRP was better than controls at the common longer follow-up (six months). The number of patients is reduced and the heterogeneity of interventions claims more research in this specific application. In a

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recent update of clinical data, we identified 15 randomized clinical studies in epicondylar tendinopathy. A common result after combination of studies is improvement in the middle term (6 months) but not in the short term [93], comparators include corticosteroids, peripheral blood, saline, anesthetic, dry-needling, physical therapy and surgery. When 10 of these studies were combined in a network metaanalysis (peripheral blood, corticosterois and PRP), results showed that both PRP and blood can improve pain and enhance function but patients treated with blood had a higher risk of adverse event [94]. The fact that epicondylopathy has free-pain periods achieved spontaneously hinders interpretation of results and enhancement of study design.

Correlations between cytokines and clinical outcomes are an emerging area of research and can help in treatment optimization. Lim et al., [95] in a controlled study including 156 patients with 24 weeks follow-up, measured PDGF-AB, -BB, TGF-β, VEGF, EGF and IL-1β, and their association with clinical and imaging outcomes was examined. TGF-β and VEGF levels increased significantly with changes in MRI grade. TGF-β significantly correlated with outcome and MRI in PRP treated patients. Accordingly, Lyras et al. [96] found enhanced expression of TGF-β in patellar tendons of rabbits treated with PRP. TGF-β is a pleiotropic factor and reduced expression of TGF-β1, TGF-βR1 and R2 compared to healthy tissue was found in pathologic rotator cuff [97].

The urge for tendinopathy treatments generates novel hypothesis. In the past years, we witnessed evolving data revealing how the microbiota shapes the immune response. In this context, recent research has identified the presence of bacteria (predominantly Staphylococcus genus) in 5 out of 20 samples obtained from spontaneous Achilles tendon ruptures during reconstructive surgery [98]; hamstring tendons used as control did not show any bacterial contamination. Intriguingly, a recent experimental study has

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reported differential PRP healing effects related to microbiota in rat Achilles tendons [99]. Taken together, these results point out the crucial role of the immune system in healing and open a new avenue for research.

Despite the socio-economic impact of tendinopathy the public awareness is low. However, new insights into the pathogenesis of tendinopathy and the optimization of PR therapies may help to trigger healing mechanism, mainly by modification of the molecular microenvironment. But PRP is not a monotherapy, it might be used in combination with cells and as part of a broader treatment program that includes load and pain management.

5. EXPERT OPINION

Tendinopathy is not a single entity, and a single etiopathogenetic hypothesis cannot fit all types and locations of tendinopathy. Development of PRP or other biological treatments is challenging because the typical group of patients with tendinopathy (age, sex, bone mass index, risk factors) does not exist as it affects multiple segments of the population with different mechanical requirements. The failed healing theory is what is currently explored as it fits with histopathological findings. However, these findings do not correlate with the clinical symptomatology, mainly pain.

A new understanding of the reason, why healing fails to resolve in tendinopathy, is needed to develop clinically meaningful biological therapies. Recent research on pathogenic mechanisms of tendinopathy has focused on immune modulation and inflammation as merging biological processes involved in healing or failed healing, Specifically, finding new methods for driving polarization of macrophages towards regenerative phenotypes is a focus of PRP research.

Failed resolution of inflammation is viewed as an active process that needs to be counteracted. In this context, the foundation of PRP is to provide supra-physiological

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concentrations of chemokines and other cytokines involved in immune regulation. In doing so, PRP can rebalance the molecular milieu within the pathological tissue, and can have an active role in resolving inflammation thereby achieve healing. Thus, in this context "medicine" is not a "drug" but the "molecular milieu" we produce. Therefore, PRP application should not be driven by the same concepts involved in drug administration, and development of application protocols (volume, number of injections, and spatial distribution of the product) is essential [100-103].

PRP is not a monotherapy but with knowledge from further studies it might be used to create an optimal macromolecular milieu for recovery/regeneration as part of a broader treatment program.

The arguments for using PRP for tendinopathy are increasing, and the field is progressing based on good focused science revealing that PRP contains tendoinductive factors, which can activate and differentiate endogenous stem cells. Improvement of PRP therapies can be achieved by creating combination products with mesenchymal cells. Actually, PRP may be able to protect stem cells to maintain their regenerative potential under severe host conditions. But not all PRP formulations are tendoinductive: thus, they should be carefully tailored according to the desired effect (L-PRP is not tenogenic). Furthermore, precise protocols for PRP application and specific guidance cues have to identify the spatial location of tendon stem cells. Not only temporal but spatial delivery merits further investigation.

Although some degree of clinical success has been achieved, PRP is not considered standard medical treatment, nor is it paid by insurance companies. The variability of PRP formulations hinders interpretation of research data and delays optimization of treatment protocols. Moreover, clinical studies show that PRP works only in selected patients. The concept that young blood is more effective than old was developed in

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parabiotic experiments with rats mainly in Alzheimer disease, and offers a new way for identification of factors that can enhance healing [104]. Using allogeneic young PRP from healthy subjects might help in some patients [105].

No clear definite pathway is strongly targeted by PRPs; thus, the identification of useful parameters for quality control has not been possible to date. The option of lyophilizing "high quality" PRPs, while preserving their biological activities, may be a promising future [106].

Unanswered questions remain mainly about the characteristics of tendinopathy within the different stages of progression, in specific anatomical areas. Progress can be based on identifying subtypes of tendinopathy, the ambition is to find out biomarker panels and/or imaging markers. This review gives hope for tendon regeneration by tailoring PRPs to intervene at various points along the pathway of the disease.

FIGURE LEGENDS

Figure 1 Converging themes in the pathophysiology are endogenous and exogenous risk factors that confer vulnerability to develop tendinopathy. Key advances in the last years consider inflammation as merging biological mechanisms crucial to the onset and perpetuation of tendinopathy.

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ABBREVIATURES

ADP adenosine di-phosphate

AKT Rac protein kinase alpha

BDNF brain derived neurotrophic factor

bFGF basic fibroblastic growth factor

CTGF connective tissue growth factor

DAMP danger associated molecular pattern

ECM extracellular matrix

HGF hepatocyte growth factor

IFN interferon

IGF insulin like growth factor

IGFBP insulin like growth factor binding protein

IL interleukin

L-PRP leukocyte rich platelet rich plasma

MCP-1 Monocyte chemoattractant protein 1

MMP metalloproteinase

MRI magnetic resonance imaging

NAP-2 neutrophil activating peptide

NFkB nuclear factor kappa B

PDGF platelet derived growth factor

PF4 platelet factor

PGE_2 prostaglandin E

PPP platelet poor plasma

Prdx1 peroxiredoxin 1

PRP platelet rich plasma

RANTES T-cell specific RANTES protein

ROS reactive oxygen species

SDF-1α stromal cell-derived factor

TGF-β transforming growth factor

TGF-βR transforming growth factor receptor

TIMP tissue inhibitory metalloproteinase

TLR Toll-like receptor

TNF-α tumor necrosis factor

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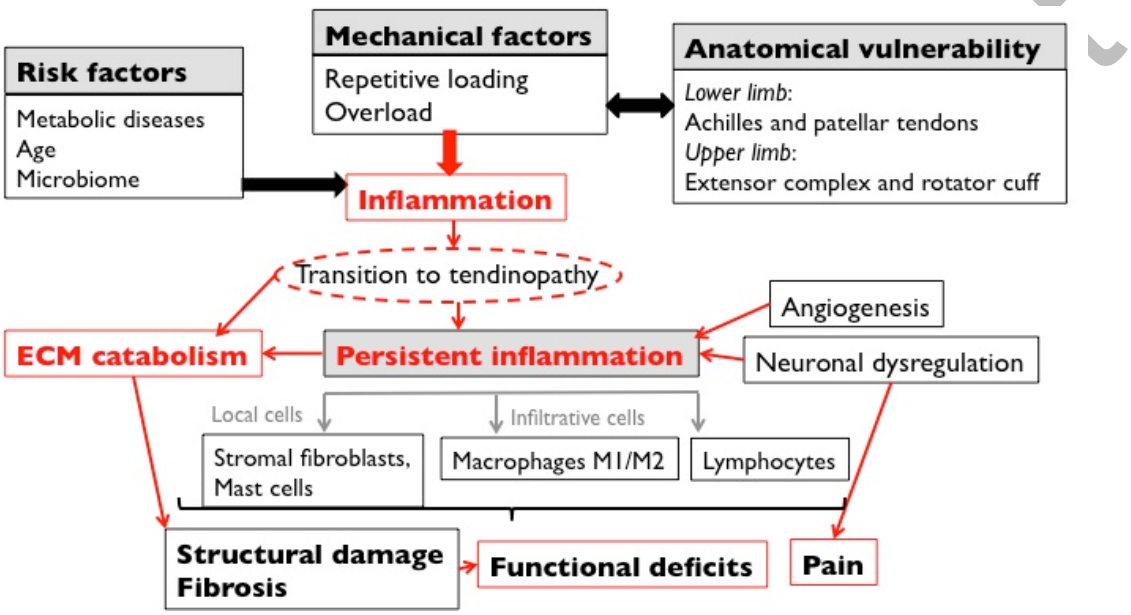
TSC tendon stem cell

TSP-1 thrombospondin 1

VEGF vascular endothelial growth factor

Figure 1:



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