Updates in biological therapies for knee injuries: tendons

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Abstract Tendons are subjected to tendinopathies caused by inflammation, degeneration, and weakening of the tendon, due to overuse and trauma, which may eventually lead to tendon rupture. Recently, there has been increasing interest in biological approaches to augment tissue healing. Tendon healing occurs through a dynamic process with inflammation, cellular proliferation, and tissue remodeling. In this review article, we discuss the more frequently proposed biological therapies for tendon injuries as platelet-rich plasma, mesenchymal stem cells, extracorporeal shockwave, and scaffolds.

Keywords Tendon • Knee • Platelet rich plasma • Biologics

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Introduction

Injuries to tendon may occur in the knee as result of acute or chronic conditions. Traumatic tendon ruptures are relatively rare in the knee. Quadriceps tendon and patellar ligament ruptures may lead to severe functional limitation if not treated. Quadriceps tendon ruptures are more frequent in patients older than 40 years and usually associated with medical conditions, such as renal disease or chronic corticosteroid injections.

Patellar tendinosis or Jumper's Knee is a frequent complaint among athletes and recreational sports practitioners. Tendinosis has been described as a failure of cell matrix adaptation to trauma resulting in imbalance between matrix synthesis and degeneration. Due to overuse and trauma, tendons are subjected to tendinopathies caused by inflammation, degeneration, and weakening of the tendon, which may eventually lead to tendon rupture. During this process there is an increased production of metalloproteinases (MMPs) that have a very important role in the degradation and remodeling cycle.

In recent years, there has been increasing interest in biological approaches to augment tissue healing. Platelet-rich plasma, mesenchymal stem cells, extracorporeal shockwave and scaffolds are the more frequent proposed biological therapies for tendon injuries. In the knee joint, research and clinical use of biological approaches in tendon injuries are mainly described in patellar tendinosis, donor site healing for patellar tendon graft, and tendon to bone healing in anterior cruciate ligament reconstruction surgery.

Platelet rich plasma

Platelet-rich plasma (PRP) is a platelet concentrate with higher growth factor and cytokine concentration, and it may be obtained by 2 different methods: centrifugation or apheresis.

Fig. 1 Apheresis PRP preparation

The centrifugation method is cheaper and simple [1]. There are several centrifugation protocols, with single [2, 3] or double centrifugation [1, 4, 5], as well as many proprietary centrifugation devices. Centrifugation should be performed using speed-forces between 200 and 400 g, avoiding speed above 700 g as platelet membranes may rupture. Different

compositions of PRP with varying platelet concentration and leukocyte concentration are produced by these techniques.

Apheresis PRP preparation results in a more constant platelet concentration, more PRP volume and less leukocytes in the final product [6] (Fig. 1). Weibrich et al demonstrated platelet concentrations above 5 times whole blood concentration with this method. The same authors also described that leukocyte concentration is 40 times below peripheral blood when using apheresis to concentrate platelets. Typical centrifugation methods increase leukocyte concentration up to 5 times. Apheresis methods also result in a preparation that has more TGF-beta 1 and less PDGF compared with PRP made by centrifugation [6]. On the other hand, apheresis is more expensive than centrifugation and requires circulation of blood to the machine and back to the patient during the filtering process.

It is well established that not all PRP preparations are the same. In order to better understand PRP research, it is important to analyze the PRP considering its final volume, platelet concentration, fibrin clot formation, and leukocyte concentration (Table 1). Ehrenfest et al (2009) proposed 4 categories: pure platelet rich plasma (P-PRP), leukocyte rich platelet rich plasma (L-PRP), pure fibrin platelet-rich (P-PRF), and leukocyte rich platelet rich fibrin (L-PRF) [12•].

The healing improving characteristics of the PRP is mainly secondary to cytokines including TGF-Beta, PDGF (platelet derived growth factor), IGF-I (insulin like growth factor-I), bFGF (basic fibroblast growth factor), VEGF (vascular endothelial growth factor, and EGF (epidermal growth factor), all released from the alpha-granules in the platelets [6, 13]. Also, it is important to remember that platelets carry dense granules with other substances as histamine, serotonin, dopamine and, catecholamines that may have a positive effect on pain control [14].

Table 1 Platelet and leukocyte concentration for different PRP preparation methods

System	Blood volume	Procedure	Time	Final volume of PRP	Final platelet concentration	Leukocytes	Activator
Haemonetics MCS +9000 / 995-E [7•]	450 mL blood returns to circulation	Apheresis		70 mL	$\times 6\text{--}\times 8$	No ($0.9 / \text{mm}^3$)	AT / CaCl_2
ACP-DS (Arthrex, Naples, FL) [8]	9 mL	Single spin	5 m	3 mL	$\times 2\text{--}\times 3$	No	None
Fibrinet (Cascade, Musculoskeletal Tissue Foundation) [9]	9–18 mL	Single spin	6 m (PRP)	4–9 mL	$\times 1\text{--}\times 1.5$	No	CaCl_2
		Double spin (for PRFM)	6+15 m (for PRFM)	membrane			
GPS (Biomet) [8]	27–110 mL	Single spin	15 m	3–12 mL	$\times 3\text{--}\times 8$	Yes	AT / CaCl_2
PRGF (BTI Biotechnology Institute) [9]	9–2 mL	Single spin	8 m	4–32 mL	$\times 2\text{--}\times 3$	Poor	CaCl_2
SmartPrep (Harvest Technologies, Plymouth, MA) [9, 10]	20–120 mL	Double spin	14 m	3–20 mL	$\times 4\text{--}\times 6$	Yes	BT / CaCl_2
Choukroun's L-PRF [11]	18–72 mL	Single spin	12 m	2–8 membranes			

Platelet-rich plasma and tendon healing

Tendon healing occurs through a dynamic process with inflammation, cellular proliferation, and tissue remodeling. Cytokines may influence healing by interfering in cell signaling, inducing cell recruiting [14, 15], and promote tissue neovascularization. IGF-I, bFbF and PDGF have a mitogenic effect on fibroblasts, stimulating cell proliferation, and recruitment. TGF-Beta and EGF act on collagen and extra-cellular matrix up to the tissue remodeling stage [16].

Kurtz et al observed better tissue healing after tendon transection in rats treated with IGF-I [17]. Chan et al observed better tissue filling, fibroblast proliferation, and collagen III synthesis in rats treated with bFGF after central third of patellar ligament removal [18]. Anaguchi et al demonstrated better biomechanical proprieties of the regenerated tissue in the central third of patellar ligament treated with TGF-beta [19]. Lyras et al reproduced Anaguchi's et al study with similar results and observed histologically superior tissue and more cellular IGF-I gene expression with PRP treatment. The authors demonstrated that growth factors improve tissue regeneration after central third patellar tendon (patellar ligament) resection in an animal study. Cellular proliferation and collagen type III synthesis improved with bFGF and tensile forces improved with TGF-beta [20, 21]. Histologic evaluation showed better tissue formation and cellular gene expression demonstrated more IGF-I with PRP treatment. Kajikawa et al observed that PRP injected to a tendon wound site promotes recruitment and activation of circulation derived cells for enhancement of the tendon healing process. The circulation-derived cells temporarily exist in wounded tissues to initiate the healing process [22].

The leukocyte concentration in PRP may influence tendon healing. In normal tendons treated with PRP ex vivo, high leukocyte concentrations increased inflammatory mediator synthesis, and decreased extracellular matrix synthesis. Preclinical studies suggest that PRP for tendon healing should be leukocyte reduced. McCarrel et al suggested that WBC in PRP contribute to inflammatory cytokine production in tendons [10]. The authors compared the effect of leukocyte-concentrated PRP, leukocyte-reduced PRP and intermediated-concentration standard PRP on digitorum superficialis tendon from young adult horses. The PRP were used as media for tendon explants in culture. Gene expression for cartilage oligomeric matrix protein (COMP), matrix metalloproteinase (MMP-13), and collagen type I (COL1A1) and type III (COL3A1) ratio were not different between PRP groups. Compared with control COMP expression is increased and MMP-13 decreased in all PRP groups. The expression of IL-1 beta was lowest in leukocyte-reduced PRP and highest in concentrated-leukocyte PRP. TNF-alpha expression was highest in concentrated leukocyte PRP [10]. In the subsequent controlled laboratory study, Bowell et al

observed that in leukocyte reduced PRP preparation, the anabolic growth factors PDGF-BB and TGF-beta1 were increased with increasing platelet concentrations, and the catabolic cytokine IL-1beta was decreased with increasing platelet concentrations but resulted in a significant reduction in COL1A1 and COL3A1 synthesis in tendons. The authors suggest that minimizing leukocytes in PRP is more important than maximizing platelet numbers with respect to decreasing inflammation and enhancing matrix gene synthesis [23].

Translational of animal studies with PRP to humans should be performed with caution as cytokine concentration, cell response to signaling, and innate healing capacity is different among animals and humans.

Platelet-rich plasma and clinical studies: tendon healing

Patellar tendinopathy

There are very few clinical studies in the knee regarding tendon healing with the use of PRP. Analogy with tendinopathies in other areas such as elbow epicondylitis, rotator cuff tears [24], and Achilles tendon PRP clinical studies are usually the basis to argue whether there is rationale in the use of PRP injections to treat patellar tendinopathy [25, 26].

Both biomechanically and histologically the patellar ligament may be different from other tendons. Patellar tendinopathy or Jumper's knee results from microtears of the patellar ligament followed by a chronic inflammatory response. Macroscopically, patellar tendinopathy contains soft, yellow-brown, and disorganized tissue, commonly described as mucoid degeneration or hyaline degeneration. Microscopically, it shows abnormal collagen, tenocytes lose their fine spindle shape, and nuclei appear more rounded. There is abundant vessel ingrowth with amorphous and disorganized collagen bundles with necrotic tendon areas. Clefts in the collagen structure generally suggest chronic microtears due to overload. The classic site of patellar tendinopathy is close to distal patella pole attachment.

Kon et al reported a case series of 20 patients with chronic patellar tendinopathy who were treated with 3 PRP injections with 15-days interval. The authors described clinical improvement and no complication in all patients [27]. Filardo et al described positive results of PRP treatment in 15 patients with chronic refractory patellar tendinopathy that had not responded to conservative treatment. The authors compared with a homogenous group of 16 patients with patellar tendinitis without previous treatment. Multiple PRP injections were performed on 3 occasions 2 weeks apart into the site of patellar tendinopathy. The authors reported statistically significant improvement in all scores at the end of the PRP injections in patients with chronic refractory patellar tendinopathy

and a further improvement was noted at 6 months, after physiotherapy was added [28].

Gosens et al evaluated the use of PRP in 36 patients with chronic patellar tendinopathy [29]. Patients were subdivided into 1 group who had undergone previous treatments for the patellar tendinopathy (ie, corticosteroids, surgery, etc) vs those who had no prior treatments. While both group of patients showed a statistically significant reduction in pain following PRP treatment, the group that had no prior treatments reported significantly better functional outcome relative to the group who had failed previous treatments (recalcitrant cases). A statistically significant improvement in all scores (Tegner score, EQ VAS, and pain level) was observed at the end of the PRP injections, and further improvement was noted with the addition of physiotherapy at 6 months [29].

Bowman et al reported 3 patients who developed progression and worsening of patellar tendinitis following PRP therapy treatment. Clinical and radiological findings following treatment included patellar tendon thickening, worsening pain, discontinuation of athletic participation in all 3 patients, and osteolysis of the distal pole of the patella in 1 patient identified during surgical intervention [30].

Dragoo et al performed a prospective randomized study in patellar tendinopathy. The authors compared the clinical outcomes after single leukocyte-rich PRP injection compared with dry needling (DN) alone. They observed a greater improvement in VISA scores at 12 weeks in PRP group, but no differences between the groups in this score at 26 weeks. Besides, they found a greater improvement in Lysholm scores in DN group at 26 weeks. The authors concluded that a single injection of leukocyte-rich PRP accelerates the recovery from patellar tendinopathy, but this benefit dissipates over time [31••].

To our knowledge there is no clinical study evaluating PRP in other knee chronic tendinopathies as iliotibial band friction syndrome, quadriceps, or femoral biceps tendinitis.

Patellar ligament graft donor site

The central third of the patella tendon is frequently a donor site for anterior cruciate ligament reconstruction and there are very few clinical studies evaluating the use of PRP in this condition. Cervellin et al performed a prospective comparative study with 40 young athletes treated with ACL reconstruction with patellar tendon grafts randomly assigned between control or autologous PRP gel applied to both the patellar and tendon bone plug harvest sites. At 12-month follow-up, VISA questionnaires scores were significantly higher in the patients treated with PRP (84.5 ± 11.8 and 97.8 ± 2.5 for group A and for group B; $P=0.041$), whereas no significant difference in postoperative VAS scores between the 2 groups was observed (1 ± 1.4 and 0.6 ± 0.9 for group A and group B, n.s.). Regarding bony tissue fill (above 70 % bone gap filled), in

85 % of PRP group patients, the tibial and patellar bone defect was satisfactorily filled, and in 60 % in control group patients (not statistically significant). De Almeida et al observed improved donor site healing with MRI evaluation in a prospective randomized double-blinded clinical trial (Fig. 2). Patellar tendon gap area was significantly smaller in the PRP group ($4.9 \pm 5.3 \text{ mm}^2$) than control (9.4 ± 4.4 , $P < 0.05 \text{ mm}^2$). There was no significant difference in clinical outcome, functional scores, or isokinetic strength evaluation. In this study PRP was obtained using an apheresis method resulting in an almost leukocyte-free product [7•].

ACL graft healing

Anterior cruciate ligament rupture is a common knee injury and usually demands surgical reconstruction. The most common grafts are central third of the patella ligament, hamstrings or tendon allografts. The graft healing process is slow and may be often incomplete [32, 33]. Research on biological approaches to improve graft healing have been proposed.

Silva and Sampaio compared 40 patients with hamstring graft double bundle ACL reconstructions in 4 situations: no PRP, PRP into bone tunnel, PRP into bone tunnel and 2 injections at 2-weeks and 4-weeks, and thrombin activated PRP into bone tunnel. The authors report no difference in tendon bone healing in 3-month MRI evaluation [34]. Nin et al compared the use of PRP with patellar tendon allograft in ACL reconstruction. The authors performed a double-blinded, randomized prospective trial with 100 patients in 2 groups (PRP and no PRP) with no statistically significant difference in pain, functional score, knee stability, or MRI evaluation [35].

Sánchez et al reported results of second-look arthroscopy and tissue biopsy in 37 autologous hamstring graft ACL reconstructions between 6-month to 2-years follow-up.

Fig. 2 PRP clot as biological augmentation in patellar ligament donor site

Twenty-two patients received PRP in the graft and fifteen patients had no PRP augmentation. The authors reported no difference in macroscopic evaluation but better tissue maturation in PRP treated ACL graft [36].

Murray et al observed enhanced histologic repair in a central wound in the anterior cruciate ligament with collagen platelet-rich plasma scaffold in an animal study. Treatment with a collagen-PRP hydrogel had significant effects on the composition of tissue filling the wound, increasing the presence of fibronectin, fibrinogen, PDGF-A, TGF-beta, FGF-2, procollagen I, and Von Willebrand Factor [37].

Radice et al reported a prospective, evaluator blinded, nonrandomized trial comparing PRP augmentation in ACL reconstructions. The authors performed MRI between 3 months and 1 year postoperatively and observed a more homogenous graft 48 % earlier in the PRP group (179 vs 369 days) [38].

Mesenchymal stem-cell and tendons

Stem cells are capable of long-term proliferation, self-renewal, and differentiation into many cell types and lineages. Mesenchymal stem cells [MSCs] are adult-derived, undifferentiated stem cells found among differentiated cells in a tissue or organ with ability to differentiate into different mesenchymal tissues, including bone, tendon, cartilage, fat, or muscle. Under appropriate culture conditions, MSCs are capable of differentiating into the osteogenic, chondrogenic, myogenic, and adipogenic lineages [39]. There is a need for better understanding of exactly how cells differentiate when applied to tissue. Further information is also required to understand if there is a paracrine effect or cell signaling effect of MSCs that may improve tissue healing [40, 41].

Tendons are relatively hypocellular with poor vascularity that creates a relatively poor healing potential [42]. Laboratory studies have shown the potential for MSCs to increase tenocyte numbers and regenerate tendon tissue. Chronic tendinopathy has worse healing potential and cell-based biological augmentation therapy may be beneficial. Nixon et al evaluated the reparative properties of adipose-derived mesenchymal stem cells in an equine collagenase-induced tendon injury model [43]. Compared with saline-treated tendons, the tendons injected with stem cells demonstrated decreased inflammation and significantly improved collagen fiber architecture and organization 6 weeks following MSC treatment. Pascual-Garrido et al evaluated the use of autologous bone marrow derived MSCs obtained by centrifugation method for chronic patellar tendinopathy in 8 patients refractory to conservative treatment [44]. The authors were able to demonstrate both structural and symptom improvements. While the majority of the gains were made in the first 12 months, the authors

reported sustained benefit following injection of MSCs out to 5 years. Regarding patient satisfaction, 7 of 8 patients said they would have the procedure again if they had the same problem in the opposite knee [44].

The role of MSCs to improve tendon graft healing in ligament reconstructive surgery has also been investigated. Li et al conducted an animal study with anterior cruciate ligament (ACL) reconstructive surgery by modifying the irradiated Achilles allograft with autogenous mesenchymal stem cells (MSCs) and PDGF-B transfected MSCs in a rabbit model. At 3, 6, and 12 weeks after surgery, histologic observation found that implantation of MSCs or PDGF-B transfected MSCs accelerated cellular infiltration into the ACL and enhanced collagen deposition in the wound. PDGF-B transfected MSCs could also lead to an initial promotion of angiogenesis. The authors proposed that this gene transfer technique or cell implantation may be a potentially useful tool for improving ligament remodeling [45]. Soon et al investigated the role of mesenchymal stem cells (MSCs) as potential agents to enhance bone tunnel and tendon healing with Achilles tendon allografts ACL reconstructions in rabbits. At 8 weeks, histologic analysis of the controls revealed the development of mature scar tissue resembling Sharpey fibers spanning the tendon-bone interface. In contrast, the MSC-enhanced reconstructions showed a mature zone of fibrocartilage blending from bone to the allograft, strongly resembling a normal ACL insertion. On biomechanical testing, the MSC-enhanced grafts had significantly higher load-to-failure rates than controls [46]. Okuizumi et al conducted a rabbit study to evaluate the effect of cell therapy with autologous synovial tissue-derived fibroblasts activated by TGF-beta1 on the necrotized ACL. The authors reported accelerated cellular infiltration and better biomechanical properties [47].

Silva et al performed a clinical study to evaluate if adult noncultivated bone marrow stem cells obtained by centrifugation could accelerate tendon-to-bone healing in the femoral tunnel, after hamstring anterior cruciate ligament (ACL) reconstruction. They performed ACL reconstruction in 43 patients prospectively randomized between adult noncultivated bone marrow stem cells and control. MRI of the knee at 3 months after surgery to evaluate the signal-to-noise ratio of the interzone showed no difference between groups [48].

Shock wave therapy and tendon healing

Shockwaves are defined as pressure waves (or transient pressure oscillations) that propagate in 3-dimensions and typically induce a clear increase in pressure within a few nanoseconds. There are very rapidly rising positive pressure impulses from 5 to 120 MPa in about 5 ns, followed by a decrease to negative pressure values of -20 MPa. Extracorporeal Shock Wave

Therapy (ESWT) was first applied in a patient in 1980 to disintegrate kidney stones and it has been proposed for treating painful plantar heel spurs, fasciitis, lateral epicondylitis, and other tendinopathies [49]. Some authors consider that pain relief promoted by shockwave is due to hyper-stimulation analgesia [50]. Currently, some researchers consider that physical ESWT may stimulate tenocyte proliferation and induction of growth factor release, including TGF β 1 and IGF-I [51].

Vulpiani et al described a case series of 73 sports patients (83 knees) with jumper's knee treated with 4 sessions of extracorporeal shock wave therapy (ESWT). After 2-years follow-up the results were satisfactory in 73.5 % of cases (54.2 % excellent results and 19.3 good results). In performing athletes (16 tendons), treatment was satisfactory in 87.5 % of cases, with an average time of resuming sport of approximately 6 weeks [52].

Vetrano et al compared platelet-rich plasma (PRP) injections and focused extracorporeal shock wave therapy (ESWT) in athletes with jumper's knee in a randomized controlled trial. Forty-six consecutive athletes with jumper's knee were selected for this study and randomized into 2 treatment groups: 2 autologous PRP injections over 2 weeks under ultrasound guidance (PRP group; $n=23$), and 3 sessions of focused extracorporeal shock wave therapy (2.400 impulses at 0.17–0.25 mJ/mm² per session) (ESWT group; $n=23$). Both groups showed clinical improvement. The PRP group showed significantly better improvement than the ESWT group in VISA-P, VAS scores at 6- and 12-month follow-up, and modified Blazina scale score at 12-month follow-up ($P<.05$ for all) [53••].

Biomaterials and scaffolds

Tissue engineered materials have been studied for tendon augmentation, to bridge or reinforce tendon repairs. These materials are either biological, synthetic, or hybrids composites. Biological scaffolds are obtained from mammalian tissue as small intestine submucosa (SIS), dermis, pericardium and tendons, where cells and antigenic material are cleaned from the tissue, and it is degraded to its constitutive molecules, mainly collagen type I. Biological scaffolds interact quickly with the host tissue and induce new tissue formation faster than synthetic scaffolds, with lower inflammatory response. The main limitations of biological scaffolds are low mechanical properties and undefined degradation rate. Biological scaffolds are usually used for augmentation of tendon repairs instead of interposition, due to its low mechanical properties. They can be combined with growth factors or PRP to increase cell migration, proliferation, and differentiation.

Gigante et al assessed the tendon regeneration ability of a collagen type I membrane in the patellar tendon defect in

rabbits. They observed well-oriented collagen fibers, and neoangiogenesis in treated lesions, compared with controls. Inflammatory reactions, leukocyte infiltration or excessive neoangiogenesis were not noted [54].

Synthetic scaffolds have higher mechanical properties, but their biocompatibility is usually poor and there is an increased risk of infection or rejection [55]. They can be either degradable or permanent, although degradable synthetic scaffolds have shown poorer results due to a high inflammatory response.

Synthetic scaffolds have higher mechanical properties, but their biocompatibility is usually poor and there is an increased risk of infection or rejection [55]. They can be either degradable or permanent, although degradable synthetic scaffolds have shown poorer results due to a high inflammatory response.

Hybrid, or biosynthetic scaffolds, match the tissue regeneration potential of biological scaffolds with the greater biomechanical properties of synthetic polymers. Meimandi-Parizi et al studied the role of collagen implant with or without a polydioxanone (PDS) sheath on the healing of a large Achilles tendon defect in rabbits. The authors observed increased biomechanical properties of the tendon, as well as an increased maturation and differentiation of collagen fibers. The collagen part of the scaffold was first degraded and the PDS part of the scaffold had a role in the alignment of the newly regenerated tissue in the defect area [56].

The combination of more than 1 biological approach, such as scaffolds with stem cells and PRP may also be a possibility to enhance all aspects of tendon healing and regeneration. PRP and growth factors have a role in cell migration and proliferation, while stem cells have a role in cell differentiation and the scaffold would provide a provisional matrix to tendon regeneration.

Conclusions

Biological therapy for tendon healing in the knee is currently under development. Although there is some promising preliminary evidence, further research is clearly required. These techniques are still considered experimental and are not indicated for routine clinical use at this time. We believe that further research will better delineate the role and benefits of these biologic augmentation techniques for tendon healing.

Compliance with Ethics Guidelines

Conflict of Interest Marco Kawamura Demange and Adriano Marques de Almeida declare that they have no conflict of interest. Scott A. Rodeo is a consultant for Rotation Medical and has stock with Cayenne Medical.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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