Review article

Growth factor delivery vehicles for tendon injuries: Mesenchymal stem cells and Platelet Rich Plasma

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Summary

***Background*: tendon tissue shows limited regeneration potential with formation of scar tissue and inferior mechanical properties. The capacity of several growth factors to improve the healing response and decrease scar formation is described in different preclinical studies. Besides the application of isolated growth factors, current research focuses on two further strategies to improve the healing response in tendon injuries: platelet rich plasma (PRP) and mesenchymal stem cells (MSCs). *Objective*: the present review focuses on these two options and describes their potential to improve tendon healing. *Results*: *in vitro* experiments and animal studies showed promising results for the use of PRP, however clinical controlled studies have shown a tendency of reduced pain related symptoms but no significant differences in overall clinical scores. On the other hand MSCs are not totally arrived in clinical use so that there is still a lack of randomized controlled trials. In basic research experiments they show an extraordinary paracrine activity, anti-inflammatory effect and the possibility to differentiate in tenocytes when different activating-factors are added. *Conclusion*: preclinical studies have shown promising results in improving tendon remodeling but the comparability of current literature is difficult due to different compositions. PRP and MSCs can act as efficient growth factor vehicles, however further studies should be performed in order to adequate investigate their clinical benefits in different tendon pathologies.**

KEY WORDS: tendon regeneration, regenerative medicine, growth factor carriers.

This study meets the ethical standards of the journal[1].

Introduction

Tendons play an essential role in the musculoskeletal system stabilizing joints and transmitting loads from muscle to bone. They show a limited regeneration potential due to their slow metabolism and limited blood supply.
During tendon healing, tenocytes produce large amounts of collagen III instead of collagen I[2]. This often results in the formation of scar tissue, representing disorganized matrix. Compared to intact tendon tissue scar tissue shows inferior mechanical properties[3].
Research within the last two decades had focus in tendon healing. Studying the molecular mechanisms had revealed the presence of several growth factors which play an essential role in the tendon healing process. Recent therapies which deliver this growth factors to the healing site have shown promising results in order to improve tendon healing[4-6].
Different *in vitro* and animal studies proved the capability of several growth factors to improve the healing response and decrease a disorganized repair[7-9]. However, until now, none of these approaches has reached clinical use in tendon repair. Furthermore, the optimal application technique is not solved yet. Recombinant growth factors show a very short half-life under physiological conditions. Thus sequential re-application is necessary to achieve sufficient growth factor levels during healing. This results in enormous costs and considerable burden for the patient.
Due to these unsolved problems regarding recombinant growth factors, researchers are presently focusing on alternative growth factor sources, namely platelet-rich plasma and autologous bone marrow concentrate. The present review focuses on both preparations and describes their potential to improve

tendon healing. It points out the rationale of their use, reviews laboratory *in vitro* and *in vivo* results, regarding their effectiveness and gives an overview of clinical studies.

Growth Factors delivery methods

Platelet rich Plasma

Per definition the term platelet-rich plasma (PRP) describes a preparation obtained from peripheral blood with enrichment of the platelet fraction[10].
Platelets produce a number of relevant cytokines participating in physiological tendon healing: platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF-1)[4,5,11,12]. In the early phase of tendon healing a key step is to increase vascularity supported by different factors[13], including VEGF and HGF. These seem to act in a dose dependent response[14]. This early angiogenic effect is vital for tendon and ligament healing[13].
The rationale behind using PRP is to stimulate platelets to secrete these anabolic growth factors, they contain. This is achieved by their activation due to their enrichment during preparation[15] (Fig. 1).

In vitro studies

PRP shows positive effects on tenocytes *in vitro*. On the one hand, it promotes cell proliferation[8], on the other it enhances cell function and stimulates the synthesis of tendon matrix[8]. During tendon healing all cytokines have different peak concentrations over time, revealed in a rat rotator cuff tendon model[4,5]. One further key factor promoting tendon healing is IGF-1. In a rabbit Achilles tendon model a peek expression of IGF-1 was found at the second week of healing in different cells, but mainly in tenocytes[5]. In the early phase of tendon healing a key step is to increase vascularity supported by different factors[13], including VEGF[16,17] and Hepatic Growth Factor (HGF). These seem to act in a dose dependent response[14] proven *in vitro*[17,18], as well as in animal models[13]. This early angiogenic effect is vital for tendon and ligament healing[13].
Furthermore, some studies even indicate, that PRP may protect tenocytes from impaired function caused by certain drugs[19-21]. Besides the reported positive effect of PRP on tenocytes, an additional antiinflammatory activity has been reported in several studies

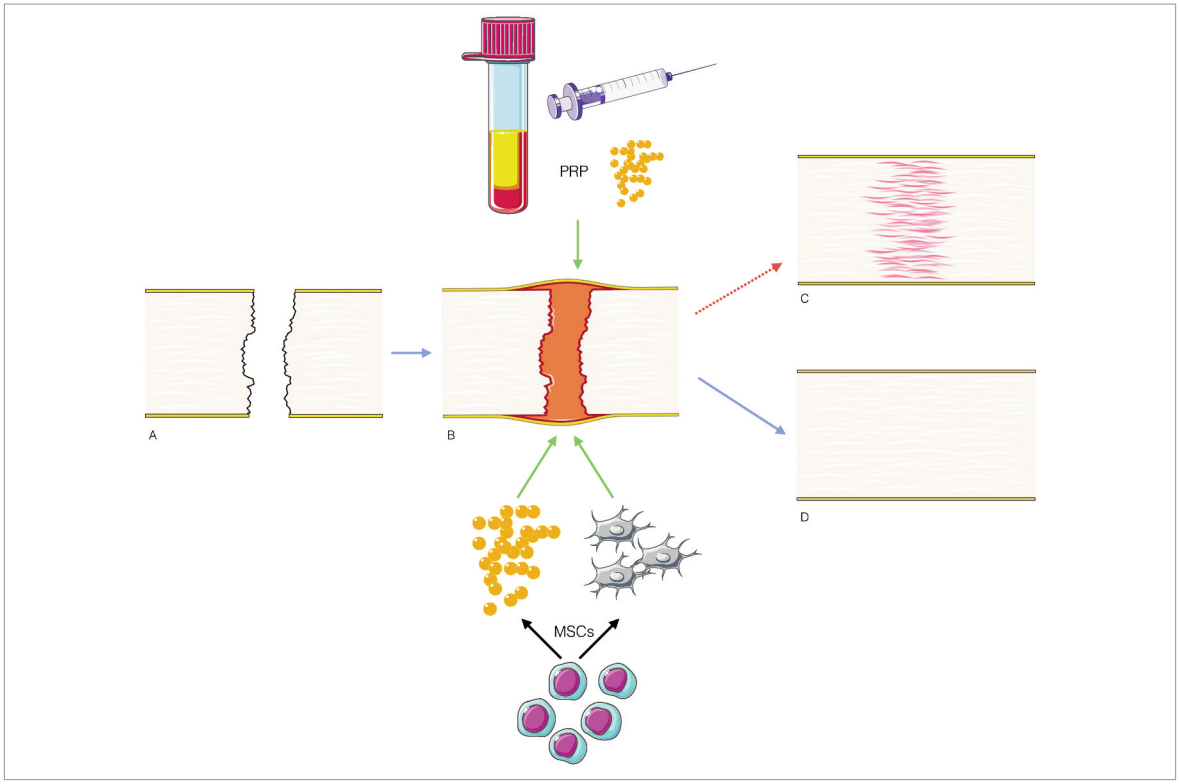

Figura 1. After a tendon injury (A) an increasing number of inflammatory cells, platelets and erythrocytes migrate to the site of injury. (B) However the remodeling phase leads to scar tissue and decreased mechanical properties. (C) Current strategies to deliver GF in order to improve the healing of the tendon (D) by direct administration of a supra physiological concentration within the PRP or the capability of MSCs either to differentiate into tenocytes or paracrine release of GF.
GF: Growth factors; PRP: Platelet rich plasma; MSCs: Mesenchymal stem cells.

attributed to the HGF[22-24]. However, the anti-inflammatory mechanism is not clarified, yet. In development of tendinopathy and during tendon healing, inflammation seems to play a key role. Here, especially prostaglandin E2 seems to be a key factor[25,26]. In this context, the anti-inflammatory effect of PRP may contribute to an improved tendon regeneration.
In vitro studies suggest also that PRP by injection does not reverse degenerative conditions of tendinopathy[27].

Animal studies

Positive effects of PRP were not only shown *in vitro* but also in animal models. In a rabbit patellar tendon model, the addition of PRP revealed increased cellular proliferation and collagen production, indicating a higher metabolic activity within the first days of tendon healing[28]. Achilles tendon bone junction shown improvement of the healing when morphogenetic protein 2 was added to PRP in a rabbit model[29]. Application of PRP resulted in superior orientation of collagen fibers and signs of increased metabolic activity in a defect model of the superficial digital flexor tendon in horses. This was also combined with a higher load to failure[30]. Beck et al. found significant effects on mechanical properties in a rotator cuff rat model by augmentation with PRP in comparison to a control group[31]. Here, improved tendon continuity was observed in histological analysis.
In conclusion animal studies revealed a positive impact of PRP on the initial healing process[32], tendon regeneration[28-30] and mechanical properties[29-31].

Human studies

Several under powered studies have reported favorable results by the use of PRP for different tendon injuries[33-40]. Several well structured clinical randomized controlled trials[41-53] report no significant differences when PRP was compared to controls for the treatment of tendinopathy (elbow, achilles, rotator cuff, plantar fascia), rotator cuff and achilles tendon ruptures.
In a systematic review and meta-analysis of controlled studies for the treatment of painful tendinopathy, Andia et al. found improvement in pain related symptoms although the studies failed to prove superiority for the PRP administration[54].

Limitations of use of PRP

To date there are several commercially available PRP systems[9,15,55], which differ among them in either the centrifugation method, platelet concentration, release rate of growth factors[9,56,57], white[58,59] and red blood cell concentration and/or activation[60]. Differences among PRP commercial presentations have not been clarified, regarding the optimal concentration of growth factors delivery and their effects on tendon regeneration.

Mesenchymal stem cells

Types and application of stem cells

Stem cells are certain cell types that are undifferentiated and inhere the ability to differentiate into different cell types. Stem cells can be found in all stages of individual development. Embryonic stem cells (ESCs) are only found in early developmental stages of the organism. They represent the only truly pluripotent cell type, having the ability to renew itself indefinitely. As a unique precursor cell, it can differentiate into cells of all three germ layers[61]. Besides the naturally occurring ESCs, researchers have demonstrated a way to dedifferentiate somatic cells into a pluripotent ESC-like status. To obtain these “induced pluripotent stem cells” (iPS) somatic cells are transfected with four embryonic transcription factors[62]. Even though ESC and iPS-cells showed promising potential in promoting tendon regeneration in preclinical studies they are far from being introduced into clinic[63]. Both cell types inhere significant cancerogenic potential[64] and ethical concerns exist towards the use of ESCs. These facts presently forbid their introduction into clinical therapies. Thus, current stem cell based concepts focus on adult stem cells. These exist in nearly all tissues of the adult body, where they are responsible to maintain the tissues integrity by substituting dying cells. In terms of tendon healing, adult stem cells from mesenchymal tissues, the mesenchymal stem cells (MSCs), are the most promising[63]. Mesenchymal stem cells are multipotent and inhere the ability to differentiate into all mesenchymal tissues, including tenocytes, osteoblasts, chondrocytes and fat cells[65].
MSCs can contribute to tendon healing in different ways[66]. First, they can provide tenocytes by direct differentiation into these cells. Secondly, they can provide a number of anabolic cytokines by their extraordinary paracrine activity. Thirdly, they show significant anti-inflammatory activity that may contribute to the healing process[63] (Fig. 1).
MSCs can be obtained from different sources[27, 66], e.g. adipose tissue (AMSCs)[67]or synovial tissue (SMSCs)[68] or the most frequent used source bone marrow (BMSCs)[69-74]. Connective tissue progenitor cells had been obtained from humeral bone marrow during rotator cuff repair in an adequate number as well as in small numbers from synovium, subacromial bursa and supraspinatus tendon[70,74].
MSCs can either be administrated by simple injection of a cell suspension (cell therapy approach), or they can be used together with biomaterials (tissue engineering approach)[63]. Here, aim of the cell therapy approach is to improve regeneration of damaged tendon tissue, as the tissue engineering approach provides new-formed tendon tissue to substitute lost tissue. This review will focus only on cell therapy approaches, as we consider tissue engineering should be discussed separately as a new and very broad topic.

In vitro studies - Mechanism of improving regeneration

Survivorship of MSCs after administration has been successfully proven by immunochemistry labeling[75,76], and differentiation of MSCs into tenocyte-like cells has been proven by several authors[77-82]. Addition of different growth factors like BMP-12[77,78], insulin[79], or viral transduction of transcription scleraxis factor[80] have successfully increased transcription of decorin[80], tenmodulin[77,80] and collagen I[80,82], and successful tenogenic differentiation when a dense collagen matrix was used[81].
Several studies revealed, that MSCs provide beneficial effects on damaged tissues without any detectable engraftment to the damaged tissue. Moreover, even protein extracts from MSCs and culture medium conditioned by MSCs could provide similar improvement in tissue function in models of liver disorders or heart ischemia, as application of MSCs[83,84]. Recent studies revealed, that these effects are mediated by the strong paracrine activity of MSCs. More and more researchers are convinced, that this paracrine stimulation of tissue regeneration is the most important mechanism of MSCs to contribute in tissue regeneration[85]. Cell proliferation, host cells protection and enhancement of angiogenesis could be attributed to this capability of MSCs to release paracrine factors like IGF-1, HGF, VEGF, IGF-2, bFGF, and pre-microRNAs[86,87].
Besides their paracrine capacity they are hypoimmunogenic and prevent T cell response as well as induce an immunosuppressive local microenvironment[88]. Therefore they may be used for immunomodulating therapies a variety of diseases or local tissue disorders. Due to their hypoimmunogenic properties they may even be used in an allogenic transplantation-approach[65,89].
In vitro studies have lead the scientific community to investigate capabilities of MSCs to improve tendon regeneration *in vivo*.

Animal studies

Several animal studies have shown the capability of MSCs to improve the tendon regeneration. Here, use of MSCs revealed particular impact on the early remodeling of the tendon-bone junction[68,90]. Regeneration of the enthesis due to MSC application was described by Nourissant et al. As they only found scar formation in the control group, a normal appearing enthesis regenerated in the MSCs group[7]. Comparable results were reported by Lim et al. who used coated tendon allografts with MSCs in a rabbit model, finding are sembling of the enthesis with cartilage in the intervening zone[91]. Here, formation of a zone of fibrocartilage blending developed on the bone to allograft surface[92]. Besides histologic improvement, application of MSCs can also enhance biomechanical properties of tendon to bone healing. This could be observed in different animal models[71,72,75,91-96].
Application of MSCs may also have a positive impact on intratendinous healing. An improved regeneration after application of MSCs could be observed in a rat achilles tendon defect model in several studies[95,97]. Furthermore, Schnabel et al. revealed positive effects of autologous MSCs in tendinitis. Using an equine tendinitis model of the flexor digitorum superficialis, the authors demonstrated significant improvement in histology[93].
Besides the potential benefits, application of MSCs may have on tendon and tendon to bone healing; some studies indicate, that MSCs may also have negative effects on regeneration. One problem in the use of MSCs is heterotopic bone formation[98]. Awad et al. used a collagen gel seeded with bone marrow MSCs to treat patella-tendon defects and after 26 weeks they found 28% of bone formations within the repair[99]. Tensile loading results in increased expression of bone morphogenetic protein 2 (BMP-2) from MSCs. This BMP2 enhances osteogenic differentiation of stem cells, providing a possible explanation for calcifying tendinopathy[100]. Also prostaglandin E2 (PGE2) is increased by mechanical stimulation. High levels of PGE2 may enhance differentiation of MSCs into adipocytes and osteocytes, instead of tenocytes[101].

Human studies

In general, application of autologous mesenchymal stem cells seems to be rather save. Up to now a significant number of patients were treated with autologous MSCs for various pathologies. Here, no severe complications, as cancerogenicy, were reported in the literature[63]. Centeno et al. investigated patients treated with ultra-expanded, autologous BMSCs, due to different orthopedic diseases. 3 cases of complication (increased swelling, pain and joint effusion) were labeled as “possible” side effect due to MSC treatment. However all of them were self-limited or regredient by conservative treatment. The study demonstrated no evidence of neoplastic complications, monitored with high field MRI tracking[102].
However until now there are only few clinical studies (Tab. 1) in the literature regarding the use of MSCs for tendon therapy. Ellera-Gomes et al. investigated application of autologous MSCs from iliac crest aspiration on rotator cuff repair. In their cohort report, they demonstrated clinical improvement and integrity of all repairs treated with MSCs[103]. Skin derived tenocyte like cells have shown satisfactory results for treatment of elbow lateral epicondylitis (Level evidence IV)[104]. Comparing the application of these cells with autologous plasma for the treatment of patellar tendinopathy, significant clinical improvement was reported (Level of evidence I), normal histology was reported in a case of a late rupture from the experimental group[105].
Although results from *in vitro* and animal studies revealed great potential of MSCs to improve tendon regeneration, the final relevance of MSCs for clinical applications in tendon therapies is not yet assessable. Here, randomized, controlled trials have to follow.

Table 1. Use of MSCs for tendon pathologies in randomized controlled trials.

Author	Study Design	Diagnosis	Source of MSCs	Comparison Group	n	Results
Clarke, et al. (2011)	Level I: Randomized double blind controlled trial	Refractory patellar tendinopathy	Skin derived tenocyte like cells	Autologous Plasma	60	Statistical difference in the cell group in clinical outcomes (VISA), No statistical difference in appearance between groups (USG).
Connell, et al. (2009)	Level IV: Prospective clinical pilot study	Refractory lateral epicondylitis	Skin derived tenocyte like cells	-	12	Improvement in pain and function in clinical outcomes (PRTEE). Tendency towards tendon normality (USG).
Ellera-Gomes, et al. (2011)	Level IV: Cohort	Rotator Cuff tear	BMMSCs	-	14	Improvement in pain and function in clinical outcomes (UCLA). Integrity of repaired tendon in all cases (MRI).

BMMSCs, Bone marrow derived mesenchymal stem cells; MRI, Magnetic resonance images; PRTEE, Patient Rated Tennis Elbow Evaluation; UCLA, the University of California at Los Angeles score;USG, ultrasonography ; VISA, Victoria Institute of Sport Assessment score.

Discussion

In the last decades plenty research has focused upon improving tendon regeneration after surgery. Despite surgical development to achieve this goal, impaired healing within the tendon continues to be a main problem in the orthopedic practice[3].

In this review both PRP and MSCs have shown to work as a carrier of growth factors into the repair site. PRP has proven to deliver growth factors *in vitro*[4,5,8,9,12,13,18,106]. These may increase angiogenesis[13,14,18] and may act as an anti-inflammatory agent[25,26]. Also MSCs have proven to deliver high amounts of growth factors due to their exceptional paracrine activity[83,84,107]. In addition, when different factors are added they inhere the potential to differentiate into tenocytes[77-82].

Animal studies revealed that both, PRP and MSCs, have the potential to improve histological and biomechanical properties of regenerated tendons[29-31,71,72,75,91-93,95,97,98]. Application of MSCs even resulted in regeneration of the enthesis in a rat model[7,91,92].

Although the benefits from PRP seemed to be encouraging in preclinical studies[28-31], PRP failed to prove significant, reproducible impact on regeneration in patients[42-46,48-52]. This is the reason why further studies are needed to improve the capabilities of the PRP use[54]. Use of MSCs in animal models are promising[7,91-93,95,97]. However, mesenchymal stem cell based therapies for tendon healing have not yet reached the patient, except of some pilot trials[102-105]. Here, randomized, controlled trials will be needed in future to investigate possible relevance and efficacy of mesenchymal stem cell therapies on tendon pathologies.

Conclusion

Current evidence shows that both, PRP and MSCs work as efficient growth factor carriers, however clinical results of PRP do not correlate with the promising preclinical results and further studies may clear the promising benefit of its use. Current evidence supports the tendon healing potential due to great MSCs characteristics, clinical results are promising, tissue engineering might prove their great features.

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