We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Mechanisms of Action of Multipotent Mesenchymal Stromal Cells in Tendon Disease

Janina Burk

Abstract

Multipotent mesenchymal stromal cells (MSCs) are a promising therapeutic tool to treat tendon disease. Aiming to establish successful treatment approaches and to fully exploit the regenerative potential of the MSC, it is crucial to understand their mechanisms of action. However, these can be multifaceted and strongly context-sensitive and are still not well-understood in the context of tendon disease. This review aims to shed light on the different possible mechanisms, including engraftment, tenogenic differentiation, extracellular matrix synthesis and remodeling, immunomodulation, pro-angiogenetic effects, trophic support, and protection of resident tendon cells. Evidence from experimental and clinical (veterinary) case studies was compiled and interpreted in conjunction with the respective in vitro and animal models used.

Keywords: MSC, ASC, progenitor cell, tendon, mechanism of action, engraftment, differentiation, extracellular matrix, remodeling, immunomodulation, trophic support

1. Introduction

Tendinopathy is a common cause of recurring pain and long-term impairment in leisure and professional athletes, increased age being an additional risk factor. The prevalence of clinically manifest conditions in risk groups is high: in a cohort of football players, 21% suffered from Achilles tendon problems [1]. Moreover, even in clinically healthy volunteers, ultrasonographic evidence of Achilles tendon alterations was found in 16% [2]. This indicates that clinical manifestation is only the tip of the iceberg, the basis of which is a long-term interplay of inflammatory and degenerative changes.

Tendons have to withstand high mechanical loads and serve as an energy storage with elastic properties. The required biomechanical properties are provided by the extracellular matrix (ECM) [3], which is largely composed of hierarchically structured, cross-linked, and crimped collagen type I fibrils. The tenocytes, while representing only 5% of the tissue volume, maintain the ECM structure by constant remodeling. This normally enables biochemical and biomechanical adaptations to exercise [4]. Recurrent overuse impairs this physiological adaptation.

The onset of tendinopathy is currently understood as the result of a failed healing response to repeated tissue trauma. Microruptures, oxidative, mechanical, and heat stress activate resident cells and trigger a cascade of inflammation

and degeneration, culminating in ECM deterioration. Key molecules involved include vascular endothelial growth factor (VEGF), interleukin (IL)-1, tumor necrosis factor (TNF)-α, prostaglandin (PG)E2, glutamate, and substance P [5, 6]. These mediators foster the ingrowth of blood vessels and nerves and the activation of nociceptive pathways. They are also implicated in the upregulation and activation of matrix metalloproteinases (MMP) and downregulation of their endogenous inhibitors (tissue inhibitors of matrix metalloproteinases; TIMP) [7]. This entails ECM degradation which successively alters and weakens the ECM structure [6]. When the accumulated damage and sensitization reach a threshold, clinical manifestation of tendinopathy comprises classical signs of inflammation including pain. Furthermore, provoked by new overload events, massive tissue trauma can occur. The resolution of inflammation is crucial to limit tissue damage, yet this mechanism often fails. Promoting fibrosis, a lack of pro-resolving signals, and persistence of macrophages entails the continuing activation of fibroblasts [8, 9]. Furthermore, macrophages could further contribute to ECM degradation via MMP secretion. Once at a diseased state, the intrinsic regenerative capacity of tendons is poor. Although endogenous mesenchymal stem-like cells with high tenogenic potential reside within tendons [10–12], these are susceptible to damage and suffer age-related changes [13, 14]. In pathological states, they could even contribute to fatty degeneration, fibrosis, and heterotopic ossifications [15, 16].

Treatment of tendinopathy still represents an unsolved challenge. Mainly, the use of strict rehabilitation exercise regimens is sufficiently evidence based [17, 18]. Anti-inflammatory drugs are frequently used, but they do not only counteract the active inflammation but also its resolution [19]. Biologicals such as platelet rich plasma have also received much attention, but clinical evidence is not convincing [17, 20, 21]. Research also focuses on the potential of endogenous tendon progenitor cells [22], which may be a promising strategy but will not be addressed in this review.

Multipotent mesenchymal stromal cells (MSCs) represent a therapeutic tool which might meet the clinical need of an adaptive treatment that simultaneously addresses different aspects of the disease. MSCs reside in virtually any tissue, in close proximity to the vasculature [23, 24]. MSCs derived from bone marrow and adipose tissue (BMSC and ASC, respectively) have been most extensively characterized [25, 26]. The fibroblast-like cells have been defined by a set of inclusion and exclusion antigens, their plastic-adherence, and trilineage differentiation potential in vitro [26]. While their differentiation potential into mesenchymal cell types, including tenocytes [27], has led to their extensive use in tissue engineering, it has become evident that their therapeutic potential by far exceeds cell replacement [24, 28]. While proof of MSC engraftment is often lacking, MSC-based cell therapy has shown beneficial effects in diverse scenarios in animal models, mostly mediated by immunomodulatory and trophic mechanisms [29–33]. Particularly, the immunomodulatory potential is extensively being researched and already exploited clinically, e.g., for treatment of graft-versus-host disease [34–36].

The use of MSC for tendon repair was first suggested in 1998 [37] and, interestingly, has been published as a case report on an equine patient as early as 2003 [38]. Since then, several experimental animal studies—the recent ones being reviewed here—and case series in equine patients [39–41] have raised hope that local implantation of MSC into acute tendon defects improves healing. However, translational progress into human orthopedics is underwhelming, and although equine patients are being treated and few first-in-man clinical trials have been performed or initiated [42–44], convincing evidence from randomized, controlled clinical studies has neither been obtained in equine nor in human patients so far [45]. This

may in part be due to our still limited understanding of the MSC mechanisms of action in tendon healing, which delays the development of targeted treatment approaches.

The aim of this review was to collect the evidence for the different possible MSC mechanisms of action in the treatment of tendon disease. In vitro and in vivo studies published within the last 5 years were screened and their results were compiled, focusing on MSC-based cell therapy using BMSC or ASC.

2. Tendon regeneration and defect models

2.1 In vitro and ex vivo models

In vitro and ex vivo models relevant to MSC mechanisms of action in tendon regeneration comprise two major groups, with some overlap (**Figure 1**). The first includes the wide range of models for tenogenic differentiation [10, 46–94]. Among these, approaches in three-dimensional dynamic cultures appear most representative for MSC mechanisms in vivo [57, 58, 64, 70, 74, 77, 79, 83, 84, 86, 87]. Typically assessed parameters following tenogenic differentiation include the expression of tenogenic transcription factors (scleraxis and, in the more recent studies, mohawk), the transmembrane glycoprotein tenomodulin, as well as the expression and deposition of extracellular matrix components (e.g., collagen I, collagen III, decorin, and tenascin-C) and biomechanical parameters in case of tissue engineered constructs. Upregulation of matrix components such as collagen I or tenascin-C and improved construct strength do not only suggest tenogenic differentiation but also indicate ECM-modulating activities of the MSC. However, it should be acknowledged that no truly specific tendon marker has yet been identified, and that only expression patterns of combined marker sets, e.g., collagen I, scleraxis, and tenascin-C, discriminate healthy tendon from diseased tendon or other musculoskeletal tissues [95].

The second group includes models investigating the interaction of MSC with tenocytes and/or the tendon ECM, using co-cultures of MSC and tenocytes, their respective conditioned media, or tendon explants [48, 69, 74, 75, 88, 91, 92, 94, 96–105].

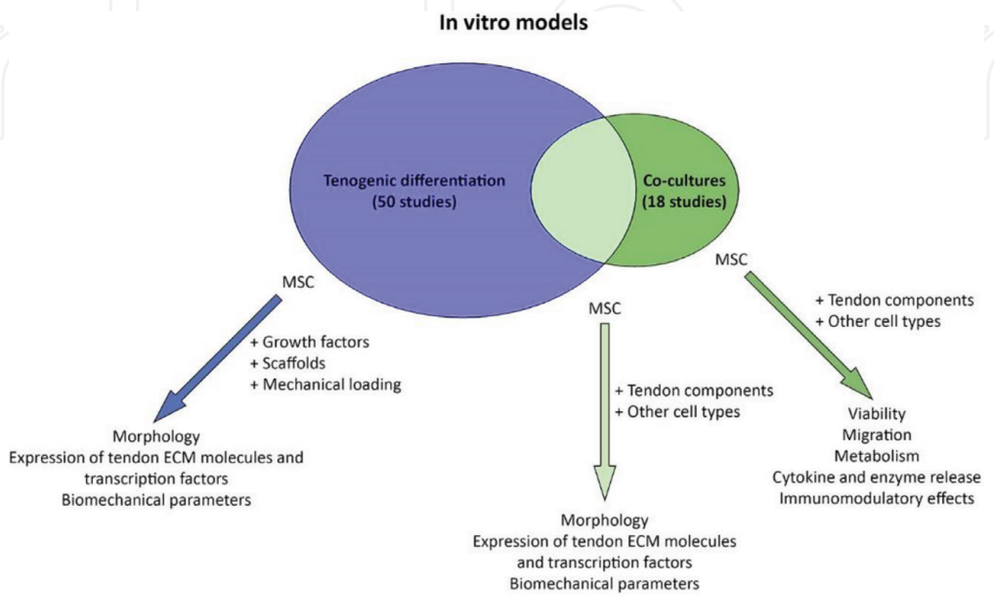

Figure 1.
In vitro models.

Outcome parameters assessed in these studies are more diverse and include cell viability, proliferation, and metabolic parameters, expression and/ or release of growth factors, cytokines, MMPs and TIMPs, expression of ECM receptors and cytoskeleton formation, ECM protein release or deposition, or modulatory effects on immune cells (e.g., macrophage M1/M2 switch). Consequently, these studies provide insight into MSC trophic effects, immunomodulatory, or matrix-modulatory mechanisms.

The figure gives an overview of the in vitro models included in this review, illustrating the overlap between tenogenic differentiation models and coculture models, and summarizes the most commonly assessed outcome parameters. Note that in this context, the term "coculture" is used to summarize the models investigating the interplay between tenocytes and MSC, thus it does not exclusively refer to cocultures of different cell types but also includes cell culture models using conditioned media or tendon explants.

2.2 In vivo models

In vivo studies on MSC-based tendon therapies need to be discriminated with respect to the animal model used (small vs. large, type of disease or defect model) and the treatment approach (strategy for MSC delivery, possible adjuvant treatments, timing of treatment, MSC source, and cell numbers applied).

Animal species used comprise small (rats [54, 106–118] and rabbits [119–122]) and large animals (dogs [123–126], sheep [127–129], and horses [130–141]). Interestingly, there appears to be a fair balance between small and large animal studies. This suggests preclinical progress, but it is also due to the interest in the equine species within the veterinary community. The tendon defects were created surgically in the majority of studies, with full thickness transections or segmental defects (mostly in the Achilles tendon) used in small animals or dogs and surgically created core lesions in the superficial digital flexor tendons in the equine model. Although there is reason to believe that enzymatical induction of tendon lesions better mimics the ECM degeneration and inflammation in tendon disease, only few among the recent studies used collagenase-based tendinopathy models [106, 108, 110, 129, 137, 139]. Still, neither surgical nor enzyme-based approaches fully reflect the complex tendon pathophysiology. In this light, providing particularly valuable information, some studies in the equine species were performed using horses suffering from naturally occurring tendinopathy [134, 138, 141] (**Figure 2**).

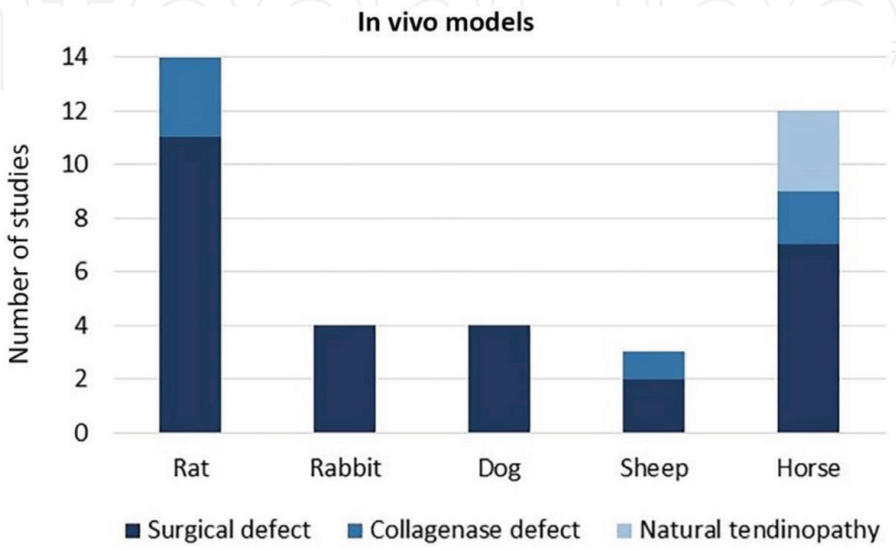

Figure 2.
In vivo models.

The diagram displays the numbers of studies performed in different animal species which were included in this review and indicates the types of tendon defect models used in the respective species.

Approaches for MSC implantation include local delivery of MSC suspensions, mostly via (ultrasound-guided) injection [106–112, 119, 120, 127–133, 136–141], coating of suture materials with MSC [113], MSC delivery in fibrin-based vehicles [54, 114, 124] or cell sheets [54, 123, 125, 126], and the use of diverse constructs of MSC and scaffold materials [115–118, 121, 122]. Interestingly, while the delineation between MSC-scaffold constructs for MSC delivery and for tendon replacement is sketchy, it is remarkable that construct-based approaches are almost exclusively used in small animals. This indicates that translational progress using these approaches is poor, possibly due to their incapability to meet the biomechanical demands in large animals or humans.

Further aspects of the treatment approach are likely to influence MSC mechanisms of action and complicate the coherent interpretation of findings from different studies. Adjuvant treatments, e.g., simultaneous growth factor delivery, or pre-treatment of the MSC, such as pre-differentiation or inflammatory licensing before cell delivery, may support certain mechanisms synergistically but may negatively interfere with other mechanisms. For example, bone morphogenetic protein (BMP)-12 promotes MSC tenogenic differentiation but reduces their immunomodulatory potential [93]. Next, the timing of the treatment is of great importance as different mechanisms of action of MSC are likely to be relevant during different stages of tendon healing. Furthermore, the dosage, i.e., the numbers of MSC applied, may not only play a role with respect to treatment efficacy but also with respect to supporting specific mechanisms of action [120]. For example, interactions between MSC and immune cells depend on the ratio of MSC to leukocytes present [142].

Last not least, the MSC source is likely to influence their mechanisms of action, which is an issue with equal relevance for in vitro findings. On the one hand, this applies to the choice of donor in terms of age and health status [143] and in terms of autologous, allogeneic or, in case of many small animal models, even xenogeneic use of MSC. On the other hand, the tissue origin of MSC as well as the donor species impact on the cell characteristics [57, 140, 144] and thus potentially on their mechanisms of action. Therefore, mainly studies focusing on the well-characterized BMSC and ASC were included and their tissue origin discriminated where appropriate. Furthermore, it was attempted to compile only studies which enabled the discrimination of MSC effects from those of possible additional treatments. In this line, in vivo studies using genetically engineered MSC for other purposes than cell tracking were not included in this review.

3. Engraftment and tenogenic differentiation

The assumption that MSC engraftment and their tenogenic differentiation after implantation into a tendon lesion lead to the replacement of damaged tenocytes dates back to the earlier days of MSC research and mirrors the general conception of MSC at that time [27, 38]. In the following years, the fact that MSC persistence at the site of tissue damage could not be achieved in models for a wide variety of diseases led to the assumption that differentiation and cell replacement might not even contribute to the regenerative effects observed after MSC transplantation [28]. This hypothesis was fostered by the compelling finding that paracrine factors released by the MSC can lead to similar beneficial effects as the MSC themselves, leading to the concept of cell-free MSC-based therapies [145]. Still, the situation

might be slightly different in tendon pathologies, and at the moment, it cannot be excluded that tenogenic differentiation of engrafted cells could contribute to regeneration, perhaps as a basis for further trophic and ECM-modulatory mechanisms.

3.1 In vitro evidence

An extensive body of recent literature describes the tenogenic differentiation of MSC in response to a wide range of stimuli, although unfortunately, no generally accepted in vitro model or standard tenogenic differentiation assay exists. Current concepts of tenogenic differentiation are reviewed in detail elsewhere [146, 147]. The most commonly used stimuli to induce tenogenesis in MSC include growth factors, scaffolds with specific topography, and cyclic mechanical loading, with most studies combining two or more of these approaches, based on earlier studies in the field of tissue engineering [37, 148–150].

Growth factors used for induction of tenogenic differentiation mainly include transforming growth factor-β family members (TGF-β [47, 51, 53, 60, 66, 86, 88] and the growth differentiation factors GDF-5/BMP-14 [60, 67, 68, 70, 82, 151], GDF-6/BMP-13 [72], GDF-7/ BMP-12 [56, 60, 80, 93], and GDF-8 [71, 78]) but also fibroblast growth factors (FGF) [49, 89, 90], insulin-like growth factor-1 [53], vascular endothelial growth factor (VEGF) [60], or epidermal growth factor [49]. A promising stepwise differentiation approach has also been reported using TGF-β1 followed by connective tissue growth factor (CTGF) [54]. Growth factors are commonly delivered as culture medium supplements, but, e.g., FGF-2-transduced MSCs have been used as well [89]. Further tenogenic differentiation approaches based on genetic modifications include the forced expression of the tenogenic transcription factors scleraxis [10, 152] or mohawk [52, 116].

Currently used scaffolds comprise decellularized tendon matrices [57, 58, 64, 65, 83, 84, 88] and (synthetic) scaffolds with specifically designed topography and stiffness [59, 61–63, 68, 70, 72–75, 79, 81, 87], both being used based on evidence that physical cues such as scaffold anisotropy and stiffness direct MSC fate. Decellularized tendon matrices provide biochemical cues at the same time. A different approach to exploit the natural tendon biochemical composition is to use tendon ECM or tenocytic extracts as a culture supplement [46, 47, 91].

Mechanical loading of cell cultures, typically MSC-seeded scaffolds, is performed in bioreactors, most commonly by uniaxial cyclic stretching [46, 57, 58, 64, 66, 70, 74, 77, 79, 83, 84, 86, 87]. Different frequencies and strain rates have been used. While results are consistent in that cyclic stretching supports tenogenic differentiation, there is a discrepancy regarding the extent of stretching, with some studies highlighting moderate strain rates of 2 or 3% as beneficial for tenogenic induction [58, 77], while others support the use of higher strain rates (e.g. 10%) [55, 153]. Further approaches to tenogenic differentiation by physical stimulation include the use of extracorporeal shock waves [76], pulsed electromagnetic fields [85], and the activation of mechanosensitive membrane receptors [50].

In addition to using growth factors, scaffolds, and mechanical loading, tenogenic differentiation of MSC has also been reported in co-cultures with tenocytes [48, 69, 74, 75, 92] or in tenocyte-conditioned medium [48].

This overview illustrates that a wide range of stimuli can induce a tenogenic phenotype in MSCs (BMSCs as well as ASCs), although the quality of differentiation cannot be directly compared between studies and certainly varies. With respect to possible MSC tenogenic differentiation in vivo, the studies relying

on physiological stimuli, such as mechanical loading, biomimetic scaffolds, or cross-talk with tenocytes, are most insightful. In contrast, the use of growth factors (typically at concentrations exceeding those found in vivo) or genetic modifications is suitable for mechanistic studies and may be helpful for tenogenic pre-differentiation prior to MSC implantation but does not reflect the in vivo situation. To understand if physiological stimuli could promote the same distinct tenogenic phenotype as artificial TGF-β concentrations, it would be helpful to gain further insight into the downstream signaling networks and their possible interfaces. So far, however, tenogenic signaling has mainly been investigated following growth factor stimulation [67, 82, 89, 90]. Only few studies have attempted to elucidate the signaling pathways activated in MSC in response to mechanical load or scaffold topographical cues, focusing on the role of rho/ROCK [154, 155].

Yet, although physiological stimuli have repeatedly been shown to induce tenogenic differentiation in MSC, it should not be anticipated that this mechanism is analogously activated when MSCs are implanted into a tendon lesion. Self-evidently, the tendon lesion does not provide a physiological but rather a pathophysiological environment, which may have an entirely different impact on the MSCs. Unfortunately, this issue is still underrepresented in the current literature. Recently, we investigated ASC tenogenic properties in response to physiological tenogenic and simultaneous inflammatory stimulation [84]. This study demonstrated that ASC tenogenic properties are compromised not only in the presence of the pro-inflammatory cytokines IL-1β and TNF-α but also in the presence of leukocytes. Similarly, IL-1β and IL-6 inhibited tenogenic differentiation in tendon-derived stem cells [156, 157]. Furthermore, again in tendon-derived stem cells, stiff matrices impeded tenogenic differentiation [158]. Together, these findings suggest that MSC tenogenic differentiation may be impaired in a pathophysiological in vivo environment, which can comprise inflammatory stimuli as well as stiff (fibrotic) ECM, depending on the stage of disease.

3.2 In vivo evidence

Although extensively investigated in vitro, there is no distinctive evidence of tenogenic differentiation following MSC implantation in vivo. One conceivable explanation is that MSC differentiation is in fact impaired in the pathophysiological lesion environment. Nevertheless, in contrast to studies in other disease models, MSCs have been repeatedly localized in treated tendon lesions, providing a basis for long-term regenerative effects, possibly including differentiation and cell replacement. Furthermore, there is some evidence of homing of MSCs to tendon lesions, although not unambiguous. The mechanism of homing may be of minor importance with respect to cell delivery at the macroscale, as the cells are almost exclusively delivered locally in MSC-based tendon therapies. Yet, the capability of homing is still indicative of MSCs that are capable of identifying regions of tissue damage at the microscale, where they would actively integrate.

None of the small animal studies included in this review specifically addressed MSC homing to tendon lesions. However, when bursal tissue was implanted in rotator cuff tendon lesions in a rat model, the green fluorescent protein-labeled mesenchymal stem cells from this tissue infiltrated the healing tendons [159], demonstrating the presence of homing signals. Accordingly, ASC infiltration into the tendons was also evident when cell sheets were used as delivery vehicle in a canine model [126]. However, when injected into the tendon sheath, BMSC homed to synovial structures but were not attracted

to the tendon lesions in an ovine model of intrasynovial tendon healing [127]. In the equine large animal model, homing of MSC to tendon lesions has been addressed in more detail. Scintigraphic short-term in vivo tracking of technetium-labeled BMSC showed that the cells homed to the tendon lesion after administration by regional limb perfusion, although local administration by direct intralesional injection was more effective, and no homing was observed after intravenous administration. These findings were consistent between artificial tendon lesions [135] and natural tendinopathy [134]. Interestingly, intraarterial limb perfusion showed greater accumulation of BMSC in the lesion on day 10 after surgical lesion induction than on day 3 [135]. This finding illustrates that the stage of tendon disease is of importance to MSC homing mechanisms. However, scintigraphic tracking also revealed that even after local injection, only a relatively small proportion of the injected BMSC remains at the injury site (24% after 24 h) [134]. In accordance with this, we and others demonstrated that ASCs are distributed via the bloodstream within the first few days after their injection into equine tendon lesions, possibly as they are washed away before they can home and attach [136, 139]. We additionally observed that the ASCs were subsequently also found in nontreated tendon lesions, indicating their capability of homing [139].

Engraftment of MSC within treated tendon lesions was demonstrated in several studies, albeit results are not conclusive as to the numbers of surviving cells in relation to the cell numbers administered. In rat Achilles tendon defects, BMSC or ASC could be identified histologically at 2, 4, and 8 weeks after cell implantation (injection) [107, 109, 112], as well as 3 weeks after implantation of a BMSC-seeded collagen scaffold [116]. Complementing these small animal studies, MSCs have been traced in large animal studies, including longitudinal in vivo cell tracking. In sheep, green or red fluorescent protein-labeled BMSCs were detected histologically at 1, 2, 3, 4, and 6 weeks following their implantation [128, 129]. In the equine model, we and others could trace superparamagnetic iron oxide-labeled ASC by magnetic resonance imaging during follow-up periods of up to 24 weeks after implantation into artificial tendon lesions [132, 139] and umbilical cord tissue-derived MSCs during a follow-up period of 8 weeks in naturally occurring tendinopathy [138]. In the experimental tendon lesions, histological results confirmed the presence of the simultaneously fluorochrome-labeled ASC until week 24 [132, 139]. This provides evidence for a remarkable long-term persistence of part of the locally injected MSC, yet it has neither been proved nor disproved whether these cells commit to a tenogenic fate.

4. Extracellular matrix modulation

The restoration of the ECM architecture and functionality is a major goal in regenerative tendon therapies. Based on the early hypothesis of MSC engraftment and tenogenic differentiation, it was assumed that the differentiated cells would subsequently synthesize new tendon ECM. Indeed, MSCs are capable to synthesize a considerable amount of extracellular matrix even in an undifferentiated state [160]. Furthermore, the composition of the ECM synthesized by differentiated MSC reflects the respective tissue lineage, which is well-established for their chondrogenic or osteogenic differentiation. Corresponding in vitro data exist for the differentiation into the tenogenic lineage, although not always consistent between studies. There is also in vivo evidence that MSC transplantation improves tendon ECM structure. However, this is not necessarily due to ECM synthesis by the MSC themselves but might also be a consequence of protective and stimulatory effects on

tenocytes, which in turn might be capable to synthesize the new ECM. Moreover, importantly, there is not simply a lack of ECM in tendinopathy but rather a dysfunctional ECM composition and structure, due to the imbalance of remodeling activities. Particularly, in later stages of the disease, chondroid degeneration and fibrosis impair ECM functionality, thus effective ECM regeneration would also comprise its remodeling and the restoration of physiological remodeling activity within the tendon.

4.1 In vitro evidence

As most tenogenic differentiation studies investigated the expression and/or deposition of tendon-specific extracellular matrix molecules as a marker for successful differentiation, there is quite extensive evidence that the ECM synthesis by MSC is altered during tenogenic differentiation. However, there is some discrepancy between different studies as to whether the ECM molecule expression pattern of tenogenic MSC truly corresponds to that of healthy tendon tissue.

Collagen I, the most abundant protein in healthy tendons, was shown to be upregulated by ectopic mohawk or scleraxis expression [52], in response to treatment with TGF-β superfamily growth factors [60, 67, 88, 93] or scaffold stiffness and alignment [61–63, 74, 81], as well as in three-dimensional dynamic cultures with uniaxial cyclic loading [58, 64, 77, 87]. Furthermore, co-culture with tenocytes in hypoxic conditions or integration of integrin-binding peptides in the scaffold increased collagen I expression on mRNA as well as protein level [69, 72]. However, in other studies, no collagen I upregulation was observed in response to growth factors such as TGF-β [49] or cyclic loading in two-dimensional ASC or BMSC cultures, respectively [66]. Data are particularly conflicting with regard to whether the presence of tendon ECM components promotes or counteracts collagen I expression [46, 47, 58, 64, 65, 83, 84, 88]. Furthermore, even if collagen I is upregulated, which would enable the MSC to contribute to tendon ECM synthesis, this often occurs in conjunction with the upregulation of other extracellular matrix molecules, such as collagen III, decorin, tenascin-C, or cartilage oligomeric matrix protein [60, 61, 69, 70, 72, 74, 77, 83]. While these molecules are important components of native tendon ECM, contributing to collagen organization and fibrillogenesis, their increased presence is also indicative of tendon degeneration or fibrosis [161–163]. Therefore, in order to achieve a beneficial ECM replacement by MSC, their ECM synthesis would have to be highly balanced. It is not yet sufficiently proven that this can be achieved by inducing tenogenic differentiation.

With respect to the hypothesis of active ECM remodeling by MSC, comparatively few data exist so far. Treatment with BMP-12 induced an enhanced secretion of MMP-1 and -8 by ASC [93]. Similarly, ASC culture in collagen scaffolds increased MMP-1, -2, -8, -9, and -13 gene expression and MMP activity compared to two-dimensional culture [46]. For tendon-derived stem cells, it was also found that cyclic mechanical loading did not only upregulate ECM-related genes but also the integrins α1, -α2, and -α11, as well as MMP-9, -13, and -14 [164]. Thus, tenogenic stimuli may increase expression and activation of MMP by MSC. Furthermore, it was found that BMSC inhibits MMP activity in the cell culture medium through secretion of TIMP-1 and TIMP-2, even in an inflammatory environment [165], but that BMSC as well as ASC accumulate active MMP at their cell surface [166]. Although these latter two studies did not focus on tendon therapies, they suggest that MSCs could contribute to matrix remodeling in a highly targeted manner.

Some studies also provide first insight into the interplay of MSC and tenocytes/tendon ECM in matrix remodeling and will therefore be addressed in more detail. In direct co-cultures of ASC and tenocytes, a different temporal regulation of MMP and ECM components was observed compared to tenocytes alone [105]. This included the upregulation of collagen I and tenascin-C gene expression at day 7 and downregulation of tenascin-C and collagen III at later time points (14 and 21 days, respectively) and a higher collagen I to collagen III ratio on protein level at day 7. MMP-1, -2 and -3, as well as TIMP-1 gene expression, increased over time in tenocytes alone but showed a different temporal regulation pattern in the co-cultures with a significantly increased MMP-3 expression at day 7 [105]. A different study from the same group investigated the indirect co-culture of ASC and tendon explants [104]. Here, total protease activity was increased in the co-cultures at day 3, as were the collagenases (putatively MMP-1 and -14) but not the stromelysins MMP-3 and -10. Furthermore, collagen III and tenascin-C deposition by ASC were reduced at day 7. Histology also suggested that ASCs had protective effects on the explant structure, but this was not consistent between donors [104]. However, seemingly in contrast to these findings, MMP-8, -9, and -13 expression by ASC in collagen scaffolds was lower upon stimulation with tendon ECM extract [46], and microvesicles from amniotic membrane mesenchymal cells induced a downregulation of MMP-1, -9, and -13 in tenocytes [101]. Thus, while it can be assumed that MSC actively contribute to and/or modulate tendon ECM remodeling, the exact temporal regulation and context-sensitivity of this mechanism need to be addressed in future studies.

4.2 In vivo evidence

Several in vivo studies have investigated the effect of MSC treatment on tendon ECM composition and structure, as well as on tendon biomechanical parameters. In most of these studies, including an equine large animal study with a follow-up of 45 weeks, the ECM composition was improved by BMSC and ASC treatment, with higher expression of collagen I on gene and/or protein level [106, 114, 120, 122, 140]. Collagen III expression was found to be decreased after ASC implantation [110, 125, 126] but increased after BMSC implantation [106, 122]. Tenascin-C and decorin were found to be increased following BMSC and ASC treatment [112, 114, 140], and glycosaminoglycans were decreased after BMSC treatment [141]. Based on these data, MSCs appear to increase collagen I deposition in healing tendons. Furthermore, as an increase of human-specific collagen I and tenascin-C was demonstrated in a rat model after human ASC implantation, there is also some evidence that MSCs actively contribute to the synthesis of new ECM [114]. The contribution of collagen III, tenascin-C, and decorin synthesis/modulation to tendon healing is to be considered controversially, as illustrated above, and certainly depends on its balance with regard to other ECM components. Yet, beyond mere collagen I synthesis, BMSC and ASC have also repeatedly been shown to improve the structural organization of healing tendons, again including the study with a 45-week follow-up, as well as an experimental trial in horses with naturally occurring tendinopathy [108, 115, 121, 140, 141]. In conjunction with the synthesis and protection of desired ECM components such as collagen I, this could be due to active ECM remodeling and the contribution of synthesized small ECM molecules to collagen fibrillogenesis. Still, it should be acknowledged that some studies in the equine model could demonstrate only few compositional or structural improvements 5 months after ASC treatment [133, 137]. Moreover, despite generally improved ECM structure and collagen I synthesis, collagen II deposits and areas staining positive for

alizarin red were found in BMSC-treated tendons [106], suggesting that erroneous MSC differentiation toward the chondrogenic and osteogenic lineage had occurred. Nevertheless, functional testing of BMSC- and ASC-treated tendons indicated an improvement of functional parameters in the majority of studies [107, 108, 112–115, 117, 119, 121, 122], which represents a beneficial effect that can be attributed to ECM regeneration [3].

So far, very few in vivo studies have investigated the effect of MSC on the presence and activation of matrix-remodeling enzymes and their endogenous inhibitors. In the equine model, MMP-13 activity was decreased 6 months after BMSC treatment [141], and MMP-3 gene expression was upregulated in the healing tendons 45 weeks after BMSC treatment [140]. Together, these results might suggest that collagen degradation could be inhibited while degradation of small ECM components is promoted. However, there is much overlap regarding MMP substrates [167], and other studies found no significant differences in MMP and TIMP expression due to ASC treatment [112]. Further studies have to substantiate this hypothesis.

When MSCs were combined with tenogenic growth factors, conflicting results were reported. Treatment with ASC and GDF-5 decreased MMP-2 and TIMP-2 expression and resulted in inferior biomechanical properties compared to ASC treatment alone [112]. Treatment with ASC and BMP-12 promoted ECM degradation, which was interpreted as a side effect of the fibrin-based delivery vehicle [124], but improved tendon ECM regeneration when delivered as cell sheets without fibrin [123]. Interestingly, the latter study showed that this may have been mediated by modulating the ECM remodeling activity of macrophages [123]. A further study from the same group demonstrated beneficial effects of combined ASC and CTGF treatment, although not evaluating effects of ASC alone [125]. A different study showed that predifferentiated BMSC sheets, induced by stepwise stimulation with TGF-β1 and CTGF, resulted in superior tendon regeneration, including improved biomechanical properties than BMSC alone [54]. However, in this study, again, fibrin was used for delivery of noninduced cells, which may have contributed to the differences observed. Thus, although some data suggest that the additional use of growth factors potentiates the beneficial effects of MSC on ECM regeneration, more evidence supporting this hypothesis is required. It should also be acknowledged that growth factor supplementation might impair other regenerative mechanisms of MSC at the same time [93].

5. Immunomodulation

There is a substantial body of evidence that demonstrates the immunomodulatory potential of MSC. While not all underlying mechanisms have been elucidated in detail yet, it is well-understood that MSCs suppress T cell proliferation and promote the modulatory M2 macrophage phenotype [168]. Furthermore, small ECM molecules synthesized by the MSC, such as tenascin-C and decorin, could contribute to immunomodulation [163, 169]. Therefore, it is likely that immunomodulation plays an important role in MSC-based tendon therapies. Against that background, it appears surprising that relatively few studies have addressed the interplay between MSC and the immune system in the context of tendon disease. This may be due to the long-existing perception that inflammation is absent during most stages of tendon disease, which, however, has been changing [5, 170]. While so far existing findings are summarized in the following, immunomodulation in the context of tendon disease will remain a promising field of future research.

5.1 In vitro evidence

In vitro evidence for MSC immunomodulation in tendon disease is scarce. The most comprehensive study investigated whether ASCs influence the effects of differently polarized macrophages on tenocytes in a tri-culture system [98]. In co-cultures of M1 macrophages and tenocytes, release of inflammatory mediators, such as PGE2 and IL-1β, was increased compared to M1 macrophage cultures alone or compared to co-cultures with M0 or M2 macrophages, suggesting inflammatory tenocyte activation. When ASCs were directly co-cultured with the macrophages for 5 days, with the tenocytes added for the last 24 h, tenocyte activation was decreased, with significantly lower release of TNF-α and IL-1β in tri-cultures with M1 macrophages. At the same time, the presence of ASC had increased CD206 expression in M0 and M1 macrophage populations, indicating a switch toward the anti-inflammatory M2 macrophage phenotype and providing insight into the suppressive mechanism. However, ASCs did not effectively counteract inflammatory activation of tenocytes by IL-1β, even when ASCs had been primed with IFN-γ [98].

Interestingly, it has also been shown that tenogenic differentiation of BMSC induced by GDF-5 involves arachidonic acid production and signaling pathways [67], suggesting a link between differentiation and inflammatory processes. In this line, addition of BMP-12 increased IL-6 secretion by ASC and attenuated the suppressive effect of ASC in a mixed lymphocyte reaction [93]. Microvesicles from amniotic membrane mesenchymal cells downregulated TNF-α expression in tenocytes but in contrast to conditioned medium, they had no effect on peripheral blood mononuclear cell proliferation [101, 171]. These studies provide preliminary insight into the modulation of inflammatory tenocyte activation by MSC, while they also suggest that their immunomodulatory potential may be higher when not tenogenically differentiated. Yet, MSC immunomodulation is highly context-specific and influenced by a variety of factors including three-dimensional culture environments as well as inflammatory priming/licensing [172, 173]. Therefore, it remains crucial to perform further studies specifically mimicking aspects of tendon pathophysiology.

5.2 In vivo evidence

The most insightful studies were performed by the same group, shedding light on ASC-mediated immunomodulation in tendon healing in the canine model [123–126]. Corresponding to the group's in vitro findings, ASC alone, delivered via cell sheets, stimulated the anti-inflammatory M2 macrophage phenotype in healing tendons and reduced total mononuclear cell infiltration. The M2 macrophage markers CD163, MRC1, and CD204 were increased on mRNA and/or protein level, as well as IL-4, prostaglandin reductase-1, and VEGF [123, 126]. Combined administration of ASC and BMP-12 promoted these effects, particularly with respect to IL-4 expression [123]. Furthermore, combined treatment with ASC and CTGF decreased IL-1β, IL-6, and IFN-γ and increased IL-4 expression [125]. These latter findings challenge the hypothesis that tenogenic differentiation decreases the MSC immunomodulatory potential. However, when the inflammatory reaction at the tendon repair site was promoted by a fibrin-based delivery vehicle, ASC and BMP-12 further fostered these unwanted effects [124]. This might indicate that strong inflammation alters the MSC immunomodulatory properties toward a proinflammatory phenotype. In contrast, priming with TNF-α increased the anti-inflammatory effects of BMSC: While nonprimed as well as primed BMSC increased IL-10 and reduced IL-1α, primed BMSC also reduced IL-12 and the numbers of M1 macrophages and increased IL-4 and the numbers of M2 macrophages in

rat Achilles tendon defects [118]. Further evidence of anti-inflammatory effects of BMSC in tendon healing was demonstrated in a rat model, in which TNF-α, IFN-γ, and IL-1β were reduced, along with an increase of IL-2 and growth factors, including VEGF [111]. Apparently in contrast to most of these findings, however, we observed that clinical signs of inflammation were increased by ASC treatment in the equine model, although this effect was transient [137]. This again illustrates that MSCs can also adopt a pro-inflammatory phenotype and raises questions as to how and whether this should be controlled. When addressing this issue, it should be acknowledged that a certain extent of inflammation is required to drive resolution. In this respect, macrophages and their M2 polarization driven by MSC may play a particularly important role.

6. Trophic support and pro-angiogenetic effects

In addition to the direct effects of MSC on ECM composition and immune cells, trophic support and protection of resident cells are likely to contribute to beneficial effects of MSC in tendon healing. Tenocytes and tendon stem cells rescued by the MSC may be enabled to promote ECM regeneration and counteract inflammation. Furthermore, a MSC-mediated increase in vascularity may be beneficial at least in some stages of tendon healing, as it would improve energy and oxygen supply, as well as disposal of metabolites, thus reduce oxidative and metabolic stress. The presence of vascular endothelial cells, as well as the combination of tenogenic growth factors with VEGF, has also been shown to promote tenogenic differentiation [60, 74]. However, increased vascularity is also associated with tendinopathy pathogenesis and may foster neurogenic inflammation [6], thus this issue is discussed controversially.

Trophic effects on tenocytes were demonstrated in vitro, when ASC and BMSC, as well as BMSC-conditioned medium, promoted the proliferation of tenocytes [94, 102, 103]. Furthermore, ASC as well as BMSC-conditioned medium promoted tenocyte migration [102, 103], and ASC promoted healing in a microwound model [92]. In vivo, results are inconsistent as to whether BMSC and ASC decrease [137, 141] or increase [117] cellularity within healing tendons. However, the rate of apoptosis was lower following BMSC treatment [107], suggesting protective effects of the MSC. Moreover, ASC combined with CTGF locally increased the numbers of CD146-positive tendon stem cells, suggesting an activation and possible rescue of this endogenous cell population [125].

Pro-angiogenetic effects were observed in small, as well as large animal studies, which demonstrated that BMSC and ASC implantation increased vascularity [106, 129, 131], likely mediated by an increase in VEGF (see below). Yet, the opposite effect was observed in horses suffering from naturally occurring tendinopathy following implantation of BMSC [141].

With respect to possible growth factor signaling, in vitro, higher TGF-β bioactivity was found in the BMSC secretome compared to tenocytes [100]. Upon tenogenic differentiation of ASC using BMP-12, VEGF secretion was significantly increased, although no effect on TGF-β was observed [93]. First in vivo evidence regarding the contribution of growth factors in tendon healing following BMSC or ASC implantation was obtained in rat models, in which VEGF, TGF-β, and hepatocyte growth factor expression were increased in the MSC treatment groups [106, 111, 112]. Yet, these studies did not comprehensively reveal whether these factors were released by the MSC or other cells within the tendon lesion.

The brevity of this subsection illustrates that the insight into trophic and protective mechanisms, as well as growth factor release by MSC, in the context of tendon

therapies is still limited. Further research is crucial to improve our ability to exploit these effects and, last not least, to prevent potential negative effects associated with some growth factors, such as hypervascularization in response to VEGF or fibrosis in response to TGF-β.

7. Discussion

This review aimed to compile the evidence supporting specific mechanisms of action that may contribute to tendon regeneration in MSC-based cellular therapies. The analysis of the recent literature demonstrated an imbalance between the numbers of studies investigating tenogenic differentiation in vitro and ECM regeneration in vivo and the numbers of studies elucidating other potential mechanisms. This is conceivable as most studies investigating MSC in the context of tendon disease did not specifically aim at clarifying the mechanisms of action. Particularly, the in vivo studies mostly addressed MSC efficacy, at which ECM characteristics are reasonable outcome parameters. Still, despite the overlap with tissue engineering, the overrepresentation of tenogenic differentiation studies may reflect a delay in the field of tendon research. Tendon pathophysiology itself is still not well-understood, making it challenging to transfer the rapidly changing perception of MSC into experimental settings relevant to tendon disease in a timely manner. Yet, it can be anticipated that the general understanding of MSC mechanisms will be successively incorporated into tendon research in the following years.

Taking into account the existing data, the best-evidenced beneficial effect of MSC in tendon regeneration is the improved ECM regeneration. MSCs may also protect and rescue resident tendon cells, but only few data support this hypothesis so far. Both, ECM regeneration and tendon cell protection, are likely to be mediated by a range of mechanisms acting in concert. These may be active over long periods of time, as the engraftment of MSC within tendon lesions was repeatedly demonstrated.

The possible mechanisms mediating ECM regeneration include ECM synthesis and targeted remodeling by the engrafted MSC, inhibition of MMP over-activation, modulation of immune cells with suppression of macrophage-mediated matrix degradation, and modulation of growth factor signaling. Last but not least, the rescue of resident tendon cells could prevent ongoing ECM degeneration, and their trophic support and stimulation by MSC-derived growth factors could re-initiate ECM synthesis and a healthy state of ECM remodeling driven by the tenocytes. A varying extent of evidence supports these different mechanisms, with the collectively most convincing data available for ECM synthesis, immunomodulation, and VEGF-mediated angiogenesis. **Figure 3** illustrates the possible interplay between the different mechanisms and their potential synergies.

The figure summarizes the currently known mechanisms of MSC that may contribute to tendon regeneration. Mechanisms for which there is conclusive evidence from in vivo studies are designated in bold typeface.

However, there may also be antagonisms between different mechanisms, although the evidence is not yet entirely conclusive. Perhaps, tenogenic differentiation and immunomodulation may not occur at the same time. Tenogenic differentiation was shown to interfere with the immunomodulatory potential of MSC [93], and inflammatory environment compromised tenogenic MSC properties [84]. Yet, some in vivo studies revealed anti-inflammatory effects of combined MSC and

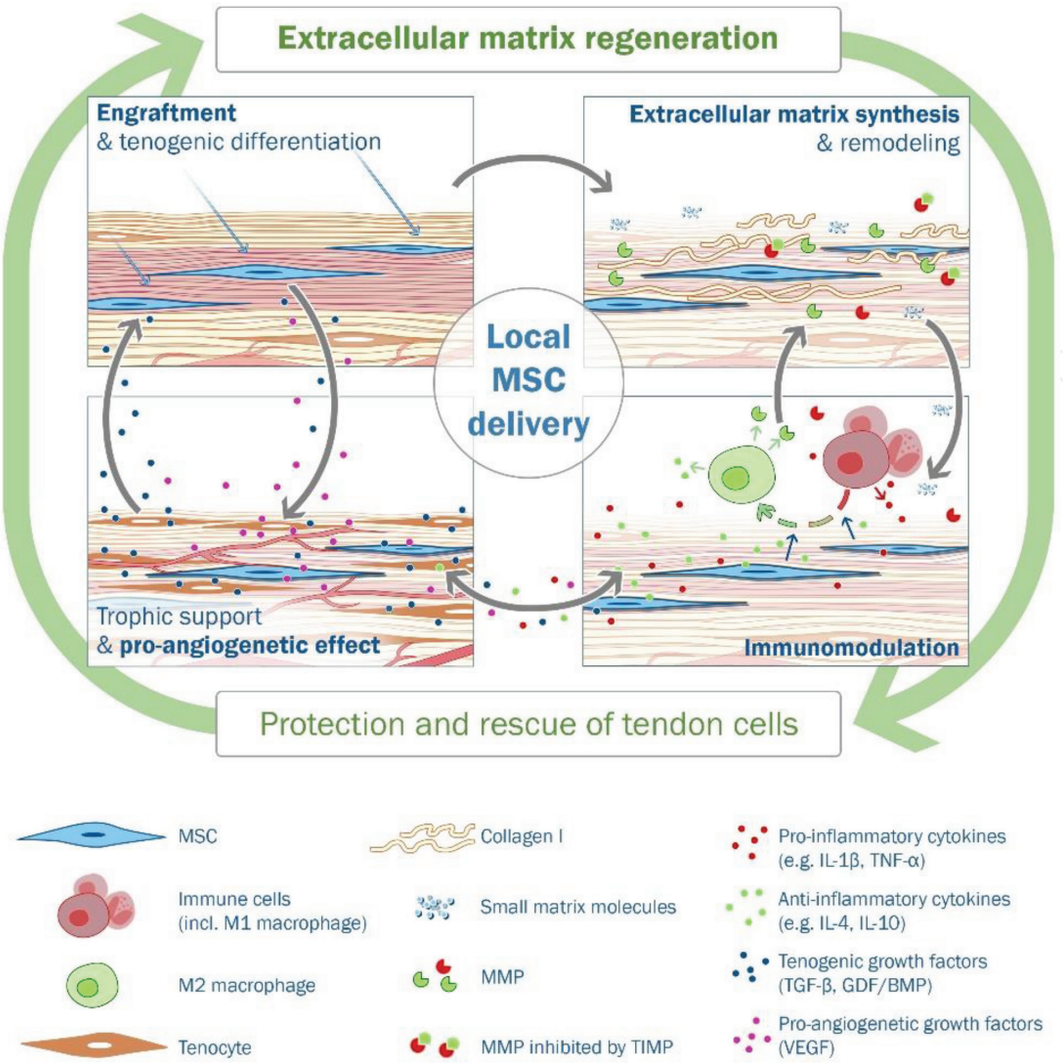

Figure 3.
Mechanisms of action of MSC in tendon healing.

tenogenic growth factor administration [123, 125], although it remained unclear if the MSCs had undergone tenogenic differentiation. It is possible that the context, i.e., the stage of tendon disease, may favor one mechanism over the other. For example, immune cells such as macrophages are not predominating during subclinical stages [6], and the macrophage polarization pattern is distinct in acute vs. chronic disease [8], which will certainly impact on the activation of MSC immunomodulatory mechanisms.

A range of limitations impedes a coherent interpretation of the existing data. These include the different treatment approaches chosen and models used, which make it difficult to elucidate specific reasons for contradictory findings. Inter-donor variability is a further issue that may obscure clarity of findings in studies using human or large animal MSCs [100, 143]. Furthermore, although tenogenic differentiation has extensively been studied, there is neither a consensus on differentiation protocols nor have specific markers for tenogenic differentiation been used consistently. Next, the limited understanding of tendon (patho)physiology makes it difficult to judge whether certain effects observed are beneficial or rather detrimental, e.g., with respect to MMP or TGF-β activity. Last but not least, the illustrated imbalance between evidence levels for particular mechanisms makes it difficult to draw a comprehensive picture at the moment.

8. Conclusion

This review demonstrates progress but also substantial weaknesses which still exist in our understanding of MSC-based cellular tendon therapy and the MSC mechanisms of action in tendon healing. Therefore, considering the low level of clinical evidence, at the moment, MSC-based treatment of tendinopathy appears only justified in the framework of clinical studies. Otherwise, although clinical translation appears temptingly close, it may be wiser to slow down the pace and focus on research into MSC mechanisms in relevant disease models to eventually be able to coax the MSCs toward targeted tendon regeneration.

Acknowledgements

I thank Dr. Wael Kafienah, University of Bristol, UK, for his input during drafting this manuscript, and Nina Schiller, Berlin, Germany, for the graphic design of **Figure 3**. This work was supported by the German Research Foundation (DFG BU3110/1-1).

Conflict of interest

The author has no conflict of interest to declare.

Author details

Janina Burk
Institute of Veterinary Physiology and Saxon Incubator for Clinical Translation, University of Leipzig, Leipzig, Germany

*Address all correspondence to: burk@rz.uni-leipzig.de

References

[1] Docking SI, Rio E, Cook J, Orchard JW, Fortington LV. The prevalence of Achilles and patellar tendon injuries in Australian football players beyond a time-loss definition. Scandinavian Journal of Medicine & Science in Sports. 2018;**28**:2016-2022. DOI: 10.1111/sms.13086

[2] Noback PC, Freibott CE, Tantigate D, Jang E, Greisberg JK, Wong T, et al. Prevalence of asymptomatic Achilles tendinosis. Foot & Ankle International. 2018;**39**:1205-1209. DOI: 10.1177/1071100718778592

[3] Hammer N, Huster D, Fritsch S, Hädrich C, Koch H, Schmidt P, et al. Do cells contribute to tendon and ligament biomechanics? PLoS One. 2014;**9**:e105037. DOI: 10.1371/journal.pone.0105037

[4] Zhang J, JH-C W. The effects of mechanical loading on tendons—An in vivo and in vitro model study. PLoS One. 2013;**8**:e71740. DOI: 10.1371/journal.pone.0071740

[5] Schulze-Tanzil G, Al-Sadi O, Wiegand E, Ertel W, Busch C, Kohl B, et al. The role of pro-inflammatory and immunoregulatory cytokines in tendon healing and rupture: New insights. Scandinavian Journal of Medicine & Science in Sports. 2011;**21**:337-351. DOI: 10.1111/j.1600-0838.2010.01265.x

[6] Abate M, Silbernagel KG, Siljeholm C, Di Iorio A, de Amicis D, Salini V, et al. Pathogenesis of tendinopathies: Inflammation or degeneration? Arthritis Research and Therapy. 2009;**11**:235. DOI: 10.1186/ar2723

[7] Del Buono A, Oliva F, Osti L, Maffulli N. Metalloproteases and tendinopathy. Muscles, Ligaments and Tendons Journal. 2013;**3**:51-57. DOI: 10.11138/mltj/2013.3.1.051

[8] Dakin SG, Werling D, Hibbert A, Abayasekara DR, Young NJ, Smith RK, et al. Macrophage sub-populations and the Lipoxin A(4) receptor implicate active inflammation during equine tendon repair. PLoS One. 2012;**7**:e32333. DOI: 10.1371/journal.pone.0032333

[9] Dakin SG, Buckley CD, Al-Mossawi MH, Hedley R, Martinez FO, Wheway K, et al. Persistent stromal fibroblast activation is present in chronic tendinopathy. Arthritis Research and Therapy. 2017;**19**:16. DOI: 10.1186/s13075-016-1218-4

[10] Hsieh C-F, Yan Z, Schumann RG, Milz S, Pfeifer CG, Schieker M, et al. In vitro comparison of 2d-cell culture and 3d-cell sheets of Scleraxis-programmed bone marrow derived mesenchymal stem cells to primary tendon stem/progenitor cells for tendon repair. International Journal of Molecular Sciences. 2018;**19**:2272. DOI: 10.3390/ijms19082272

[11] Tempfer H, Wagner A, Gehwolf R, Lehner C, Tauber M, Resch H, et al. Perivascular cells of the supraspinatus tendon express both tendon- and stem cell-related markers. Histochemistry and Cell Biology. 2009;**131**:733-741. DOI: 10.1007/s00418-009-0581-5

[12] Bi Y, Ehirchiou D, Kilts TM, Inkson CA, Embree MC, Sonoyama W, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nature Medicine. 2007;**13**:1219-1227

[13] Xu H, Liu F. Downregulation of FOXP1 correlates with tendon stem/progenitor cells aging. Biochemical and Biophysical Research Communications. 2018;**504**:96-102. DOI: 10.1016/j.bbrc.2018.08.136

[14] Gehwolf R, Wagner A, Tempfer H, Tauber M, Bauer H-C. Tendon progenitor cells - their appearance and distribution in degenerated and ageing tendon. Journal of Stem Cells & Regenerative Medicine. 2010;**6**:129

[15] Jensen AR, Kelley BV, Mosich GM, Ariniello A, Eliasberg CD, Vu B, et al. Neer award 2018: Platelet-derived growth factor receptor α co-expression typifies a subset of platelet-derived growth factor receptor β-positive progenitor cells that contribute to fatty degeneration and fibrosis of the murine rotator cuff. Journal of Shoulder and Elbow Surgery. 2018;**27**:1149-1161. DOI: 10.1016/j.jse.2018.02.040

[16] Agarwal S, Loder SJ, Cholok D, Peterson J, Li J, Breuler C, et al. Scleraxis-lineage cells contribute to ectopic bone formation in muscle and tendon. Stem Cells (Dayton, Ohio). 2017;**35**:705-710. DOI: 10.1002/stem.2515

[17] Abat F, Alfredson H, Cucchiarini M, Madry H, Marmotti A, Mouton C, et al. Current trends in tendinopathy: Consensus of the ESSKA basic science committee. Part II: Treatment options. Journal of Experimental Orthopaedics. 2018;**5**:38. DOI: 10.1186/s40634-018-0145-5

[18] Abat F, Alfredson H, Cucchiarini M, Madry H, Marmotti A, Mouton C, et al. Current trends in tendinopathy: Consensus of the ESSKA basic science committee. Part I: Biology, biomechanics, anatomy and an exercise-based approach. Journal of Experimental Orthopaedics. 2017;**4**:18. DOI: 10.1186/s40634-017-0092-6

[19] Dakin SG, Dudhia J, Smith RKW. Science in brief: Resolving tendon inflammation. A new perspective. Equine Veterinary Journal. 2013;**45**:398-400. DOI: 10.1111/evj.12030

[20] Krogh TP, Ellingsen T, Christensen R, Jensen P, Fredberg U. Ultrasound-guided injection therapy of Achilles tendinopathy with platelet-rich plasma or saline: A randomized, blinded, placebo-controlled trial. The American Journal of Sports Medicine. 2016;**44**:1990-1997. DOI: 10.1177/0363546516647958

[21] Di Matteo B, Filardo G, Kon E, Marcacci M. Platelet-rich plasma: Evidence for the treatment of patellar and Achilles tendinopathy—A systematic review. Musculoskeletal Surgery. 2015;**99**:1-9. DOI: 10.1007/s12306-014-0340-1

[22] Li Y, Dai G, Shi L, Lin Y, Chen M, Li G, et al. The potential roles of tendon stem/progenitor cells in tendon ageing. Current Stem Cell Research and Therapy. 2016;**14**(1):34-42. DOI: 10.2174/1574888X13666181017112233

[23] da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. Journal of Cell Science. 2006;**119**:2204-2213

[24] Caplan AI. New MSC: MSCs as pericytes are sentinels and gatekeepers. Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society. 2017;**35**:1151-1159. DOI: 10.1002/jor.23560

[25] Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;**15**:641-648. DOI: 10.1016/j.jcyt.2013.02.006

[26] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**:315-317. DOI: 10.1080/14653240600855905

[27] Caplan AI, Bruder SP. Mesenchymal stem cells: Building blocks for molecular medicine in the 21st century. Trends in Molecular Medicine. 2001;**7**:259-264

[28] Caplan AI. Why are MSCs therapeutic? New data: New insight. The Journal of Pathology. 2009;**217**:318-324. DOI: 10.1002/path.2469

[29] Berebichez-Fridman R, Gómez-García R, Granados-Montiel J, Berebichez-Fastlicht E, Olivos-Meza A, Granados J, et al. The holy grail of Orthopedic surgery: Mesenchymal stem cells-their current uses and potential applications. Stem Cells International. 2017;**2017**:2638305. DOI: 10.1155/2017/2638305

[30] Jeong H, Yim HW, Park H-J, Cho Y, Hong H, Kim NJ, et al. Mesenchymal stem cell therapy for ischemic heart disease: Systematic review and meta-analysis. International Journal of Stem Cells. 2018;**11**:1-12. DOI: 10.15283/ijsc17061

[31] Laroni A, de Rosbo NK, Uccelli A. Mesenchymal stem cells for the treatment of neurological diseases: Immunoregulation beyond neuroprotection. Immunology Letters. 2015;**168**:183-190. DOI: 10.1016/j.imlet.2015.08.007

[32] Fitzsimmons REB, Mazurek MS, Soos A, Simmons CA. Mesenchymal stromal/stem cells in regenerative medicine and tissue engineering. Stem Cells International. 2018;**2018**:8031718. DOI: 10.1155/2018/8031718

[33] Galipeau J, Sensébé L. Mesenchymal stromal cells: Clinical challenges and therapeutic opportunities. Cell Stem Cell. 2018;**22**:824-833. DOI: 10.1016/j.stem.2018.05.004

[34] Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: An update. Cell Transplantation. 2016;**25**:829-848. DOI: 10.3727/096368915X689622

[35] Wang L-T, Ting C-H, Yen M-L, Liu K-J, Sytwu H-K, Wu KK, et al. Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: Review of current clinical trials. Journal of Biomedical Science. 2016;**23**:76. DOI: 10.1186/s12929-016-0289-5

[36] Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet. 2008;**371**:1579-1586. DOI: 10.1016/S0140-6736(08)60690-X

[37] Young RG, Butler DL, Weber W, Caplan AI, Gordon SL, Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. Journal of Orthopaedic Research. 1998;**16**:406-413

[38] Smith RK, Korda M, Blunn GW, Goodship AE. Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment. Equine Veterinary Journal. 2003;**35**:99-102

[39] Godwin EE, Young NJ, Dudhia J, Beamish IC, Smith RK. Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon. Equine Veterinary

Journal. 2012;**44**:25-32. DOI: 10.1111/j.2042-3306.2011.00363.x

[40] Pacini S, Spinabella S, Trombi L, Fazzi R, Galimberti S, Dini F, et al. Suspension of bone marrow-derived undifferentiated mesenchymal stromal cells for repair of superficial digital flexor tendon in race horses. Tissue Engineering. 2007;**13**:2949-2955

[41] Smith RK, Webbon PM. Harnessing the stem cell for the treatment of tendon injuries: Heralding a new dawn? Br. The Journal of Sports Medicine. 2005;**39**:582-584

[42] Goldberg AJ, Zaidi R, Brooking D, Kim L, Korda M, Masci L, et al. Autologous stem cells in Achilles tendinopathy (ASCAT): Protocol for a phase IIA, single-centre, proof-of-concept study. BMJ Open. 2018;**8**:e021600. DOI: 10.1136/bmjopen-2018-021600

[43] Kim YS, Sung CH, Chung SH, Kwak SJ, Koh YG. Does an injection of adipose-derived mesenchymal stem cells loaded in fibrin glue influence rotator cuff repair outcomes? A clinical and magnetic resonance imaging study. The American Journal of Sports Medicine. 2017;**45**:2010-2018. DOI: 10.1177/0363546517702863

[44] Jo CH, Chai JW, Jeong EC, Oh S, Kim PS, Yoon JY, et al. Intratendinous injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of rotator cuff disease: A first-in-human trial. Stem Cells (Dayton, Ohio). 2018;**36**:1441-1450. DOI: 10.1002/stem.2855

[45] Pas HIMFL, Moen MH, Haisma HJ, Winters M. No evidence for the use of stem cell therapy for tendon disorders: A systematic review. British Journal of Sports Medicine. 2017;**51**:996-1002. DOI: 10.1136/bjsports-2016-096794

[46] Yang G, Rothrauff BB, Lin H, Gottardi R, Alexander PG, Tuan RS. Enhancement of tenogenic differentiation of human adipose stem cells by tendon-derived extracellular matrix. Biomaterials. 2013;**34**:9295-9306. DOI: 10.1016/j.biomaterials.2013.08.054

[47] Yang G, Rothrauff BB, Lin H, Yu S, Tuan RS. Tendon-derived extracellular matrix enhances transforming growth factor-β3-induced tenogenic differentiation of human adipose-derived stem cells. Tissue Engineering Parts A. 2017;**23**:166-176. DOI: 10.1089/ten.TEA.2015.0498

[48] Kraus A, Woon C, Raghavan S, Megerle K, Pham H, Chang J. Co-culture of human adipose-derived stem cells with tenocytes increases proliferation and induces differentiation into a tenogenic lineage. Plastic and Reconstructive Surgery. 2013;**132**:754e-766e. DOI: 10.1097/PRS.0b013e3182a48b46

[49] Goncalves AI, Rodrigues MT, Lee SJ, Atala A, Yoo JJ, Reis RL, et al. Understanding the role of growth factors in modulating stem cell tenogenesis. PLoS One. 2013;**8**:e83734. DOI: 10.1371/journal.pone.0083734

[50] Gonçalves AI, Rotherham M, Markides H, Rodrigues MT, Reis RL, Gomes ME, et al. Triggering the activation of Activin a type II receptor in human adipose stem cells towards tenogenic commitment using mechanomagnetic stimulation. Nanomedicine: Nanotechnology, Biology and Medicine. 2018;**14**:1149-1159. DOI: 10.1016/j.nano.2018.02.008

[51] Gonçalves AI, Gershovich PM, Rodrigues MT, Reis RL, Gomes ME. Human adipose tissue-derived tenomodulin positive subpopulation of stem cells: A promising source of tendon progenitor cells. Journal of Tissue

Engineering and Regenerative Medicine. 2018;**12**:762-774. DOI: 10.1002/term.2495

[52] Liu H, Zhang C, Zhu S, Lu P, Zhu T, Gong X, et al. Mohawk promotes the tenogenesis of mesenchymal stem cells through activation of the TGFbeta signaling pathway. Stem Cells (Dayton, Ohio). 2015;**33**:443-455. DOI: 10.1002/stem.1866

[53] Cong XX, Rao XS, Lin JX, Liu XC, Zhang GA, Gao XK, et al. Activation of AKT-mTOR Signaling directs Tenogenesis of mesenchymal stem cells. Stem Cells (Dayton, Ohio). 2018;**36**: 527-539. DOI: 10.1002/stem.2765

[54] Yin Z, Guo J, Wu T-Y, Chen X, Xu L-L, Lin S-E, et al. Stepwise differentiation of mesenchymal stem cells augments tendon-like tissue formation and defect repair In vivo. Stem Cells Translational Medicine. 2016;**5**:1106-1116. DOI: 10.5966/sctm.2015-0215

[55] Nam HY, Pingguan-Murphy B, Amir Abbas A, Mahmood Merican A, Kamarul T. The proliferation and tenogenic differentiation potential of bone marrow-derived mesenchymal stromal cell are influenced by specific uniaxial cyclic tensile loading conditions. Biomechanics and Modeling in Mechanobiology. 2015;**14**:649-663. DOI: 10.1007/s10237-014-0628-y

[56] Stanco D, Vigano M, Perucca Orfei C, Di Giancamillo A, Peretti GM, Lanfranchi L, et al. Multidifferentiation potential of human mesenchymal stem cells from adipose tissue and hamstring tendons for musculoskeletal cell-based therapy. Regenerative Medicine. 2015;**10**:729-743. DOI: 10.2217/rme.14.92

[57] Youngstrom DW, LaDow JE, Barrett JG. Tenogenesis of bone marrow-, adipose-, and tendon-derived stem cells in a dynamic bioreactor. Connective Tissue Research. 2016:1-12. DOI: 10.3109/03008207.2015.1117458

[58] Youngstrom DW, Rajpar I, Kaplan DL, Barrett JG. A bioreactor system for in vitro tendon differentiation and tendon tissue engineering. Journal of Orthopaedic Research. 2015;**33**:911-918. DOI: 10.1002/jor.22848

[59] Iannone M, Ventre M, Formisano L, Casalino L, Patriarca EJ, Netti PA. Nanoengineered surfaces for focal adhesion guidance trigger mesenchymal stem cell self-organization and tenogenesis. Nano Letters. 2015;**15**: 1517-1525. DOI: 10.1021/nl503737k

[60] Bottagisio M, Lopa S, Granata V, Talò G, Bazzocchi C, Moretti M, et al. Different combinations of growth factors for the tenogenic differentiation of bone marrow mesenchymal stem cells in monolayer culture and in fibrin-based three-dimensional constructs. Differentiation; Research in Biological Diversity. 2017;**95**:44-53. DOI: 10.1016/j.diff.2017.03.001

[61] Islam A, Mbimba T, Younesi M, Akkus O. Effects of substrate stiffness on the tenoinduction of human mesenchymal stem cells. Acta Biomaterialia. DOI: 10.1016/j.actbio.2017.05.058

[62] Islam A, Younesi M, Mbimba T, Akkus O. Collagen substrate stiffness anisotropy affects cellular elongation, nuclear shape, and stem cell fate toward anisotropic tissue lineage. Advanced Healthcare Materials. 2016;**5**:2237-2247. DOI: 10.1002/adhm.201600284

[63] Younesi M, Islam A, Kishore V, Anderson JM, Akkus O. Tenogenic induction of human MSCs by anisotropically aligned collagen biotextiles. Advanced Functional Materials. 2014;**24**:5762-5770. DOI: 10.1002/adfm.201400828

[64] Qin T-W, Sun Y-L, Thoreson AR, Steinmann SP, Amadio PC, An K-N, et al. Effect of mechanical stimulation on bone marrow stromal cell-seeded tendon slice constructs: A potential engineered tendon patch for rotator cuff repair. Biomaterials. 2015;**51**: 43-50. DOI: 10.1016/j.biomaterials.2015.01.070

[65] Ning L-J, Zhang Y-J, Zhang Y, Qing Q, Jiang Y-L, Yang J-L, et al. The utilization of decellularized tendon slices to provide an inductive microenvironment for the proliferation and tenogenic differentiation of stem cells. Biomaterials. 2015;**52**:539-550. DOI: 10.1016/j.biomaterials.2015.02.061

[66] Brown JP, Galassi TV, Stoppato M, Schiele NR, Kuo CK. Comparative analysis of mesenchymal stem cell and embryonic tendon progenitor cell response to embryonic tendon biochemical and mechanical factors. Stem Cell Research & Therapy. 2015;**6**:89. DOI: 10.1186/s13287-015-0043-z

[67] Tan S-L, Ahmad TS, Ng W-M, Azlina AA, Azhar MM, Selvaratnam L, et al. Identification of pathways mediating growth differentiation factor5-induced tenogenic differentiation in human bone marrow stromal cells. PLoS One. 2015;**10**:e0140869. DOI: 10.1371/journal.pone.0140869

[68] Vuornos K, Bjorninen M, Talvitie E, Paakinaho K, Kellomaki M, Huhtala H, et al. Human adipose stem cells differentiated on braided polylactide scaffolds is a potential approach for tendon tissue engineering. Tissue Engineering Parts A. 2016;**22**:513-523. DOI: 10.1089/ten.tea.2015.0276

[69] Yu Y, Zhou Y, Cheng T, Lu X, Yu K, Zhou Y, et al. Hypoxia enhances tenocyte differentiation of adipose-derived mesenchymal stem cells by inducing hypoxia-inducible factor-1α in a co-culture system. Cell Proliferation. 2016;**49**:173-184. DOI: 10.1111/cpr.12250

[70] Govoni M, Berardi AC, Muscari C, Campardelli R, Bonafè F, Guarnieri C, et al. An engineered multiphase three-dimensional microenvironment to ensure the controlled delivery of cyclic strain and human growth differentiation factor 5 for the tenogenic commitment of human bone marrow mesenchymal stem cells. Tissue Engineering Parts A. 2017;**23**:811-822. DOI: 10.1089/ten.TEA.2016.0407

[71] Le W, Yao J. The effect of myostatin (GDF-8) on proliferation and tenocyte differentiation of rat bone marrow-derived mesenchymal stem cells. The journal of hand surgery Asian-Pacific Volume. 2017;**22**:200-207. DOI: 10.1142/S0218810417500253

[72] Rehmann MS, Luna JI, Maverakis E, Kloxin AM. Tuning microenvironment modulus and biochemical composition promotes human mesenchymal stem cell tenogenic differentiation. Journal of Biomedical Materials Research. Part A. 2016;**104**:1162-1174. DOI: 10.1002/jbm.a.35650

[73] Laranjeira M, Domingues RMA, Costa-Almeida R, Reis RL, Gomes ME. 3D mimicry of native-tissue-fiber architecture guides tendon-derived cells and adipose stem cells into artificial tendon constructs. Small (Weinheim an der Bergstrasse, Germany). 2017;**13**(31):1700689. DOI: 10.1002/smll.201700689

[74] Wu S, Wang Y, Streubel PN, Duan B. Living nanofiber yarn-based woven biotextiles for tendon tissue engineering using cell tri-culture and mechanical stimulation. Acta Biomaterialia. 2017;**62**:102-115. DOI: 10.1016/j.actbio.2017.08.043

[75] Wu S, Peng H, Li X, Streubel PN, Liu Y, Duan B. Effect of scaffold morphology and cell co-culture on

tenogenic differentiation of HADMSC on centrifugal melt electrospun poly (L-lactic acid) fibrous meshes. Biofabrication. 2017;**9**:44106. DOI: 10.1088/1758-5090/aa8fb8

[76] Rinella L, Marano F, Paletto L, Fraccalvieri M, Annaratone L, Castellano I, et al. Extracorporeal shock waves trigger tenogenic differentiation of human adipose-derived stem cells. Connective Tissue Research. 2018:1-13. DOI: 10.1080/03008207.2018.1424147

[77] Subramanian G, Stasuk A, Elsaadany M, Yildirim-Ayan E. Effect of uniaxial tensile cyclic loading regimes on matrix organization and tenogenic differentiation of adipose-derived stem cells encapsulated within 3D collagen scaffolds. Stem Cells International. 2017;**2017**:6072406. DOI: 10.1155/2017/6072406

[78] Le W, Cheah AE-J, Yao J. Ex-vivo tendon repair augmented with bone marrow derived mesenchymal stem cells stimulated with myostatin for tenogenesis. The journal of hand surgery Asian-Pacific Volume. 2018;**23**:47-57. DOI: 10.1142/S2424835518500066

[79] Morita Y, Yamashita T, Toku T, Ju Y. Optimization of differentiation time of mesenchymal-stem-cell to tenocyte under a cyclic stretching with a microgrooved culture membrane and selected measurement cells. Acta of Bioengineering and Biomechanics. 2018;**20**:3-10

[80] Viganò M, Perucca Orfei C, de Girolamo L, Pearson JR, Ragni E, de Luca P, et al. Housekeeping gene stability in human mesenchymal stem and tendon cells exposed to tenogenic factors. Tissue Engineering. Part C, Methods. 2018;**24**:360-367. DOI: 10.1089/ten.TEC.2017.0518

[81] Zhou K, Feng B, Wang W, Jiang Y, Zhang W, Zhou G, et al. Nanoscaled and microscaled parallel topography promotes tenogenic differentiation of ASC and neotendon formation in vitro. International Journal of Nanomedicine. 2018;**13**:3867-3881. DOI: 10.2147/IJN.S161423

[82] Wang D, Jiang X, Lu A, Tu M, Huang W, Huang P. BMP14 induces tenogenic differentiation of bone marrow mesenchymal stem cells in vitro. Experimental and Therapeutic Medicine. 2018;**16**:1165-1174. DOI: 10.3892/etm.2018.6293

[83] Burk J, Plenge A, Brehm W, Heller S, Pfeiffer B, Kasper C. Induction of tenogenic differentiation mediated by extracellular tendon matrix and short-term cyclic stretching. Stem Cells International. 2016;**2016**:7342379. DOI: 10.1155/2016/7342379

[84] Brandt L, Schubert S, Scheibe P, Brehm W, Franzen J, Gross C, et al. Tenogenic properties of mesenchymal progenitor cells are compromised in an inflammatory environment. International Journal of Molecular Sciences. 2018;**19**(9):2549. DOI: 10.3390/ijms19092549

[85] Marmotti A, Peretti GM, Mattia S, Mangiavini L, de Girolamo L, Viganò M, et al. Pulsed electromagnetic fields improve tenogenic commitment of umbilical cord-derived mesenchymal stem cells: A potential strategy for tendon repair-an in vitro study. Stem Cells International. 2018;**2018**:9048237. DOI: 10.1155/2018/9048237

[86] Zhang B, Luo Q, Deng B, Morita Y, Ju Y, Song G. Construction of tendon replacement tissue based on collagen sponge and mesenchymal stem cells by coupled mechano-chemical induction and evaluation of its tendon repair abilities. Acta Biomaterialia. 2018;**74**:247-259. DOI: 10.1016/j.actbio.2018.04.047

[87] Bosworth LA, Rathbone SR, Bradley RS, Cartmell SH. Dynamic

loading of electrospun yarns guides mesenchymal stem cells towards a tendon lineage. Journal of the Mechanical Behavior of Biomedical Materials. 2014;**39**:175-183. DOI: 10.1016/j.jmbbm.2014.07.009

[88] Roth SP, Schubert S, Scheibe P, Groß C, Brehm W, Burk J. Growth factor-mediated tenogenic induction of multipotent mesenchymal stromal cells is altered by the microenvironment of tendon matrix. Cell Transplantation. 2018;**27**:1434-1450. DOI: 10.1177/0963689718792203

[89] Cai T-Y, Zhu W, Chen X-S, Zhou S-Y, Jia L-S, Sun Y-Q. Fibroblast growth factor 2 induces mesenchymal stem cells to differentiate into tenocytes through the MAPK pathway. Molecular Medicine Reports. 2013;**8**:1323-1328. DOI: 10.3892/mmr.2013.1668

[90] Reed SA, Johnson SE. Expression of scleraxis and tenascin C in equine adipose and umbilical cord blood derived stem cells is dependent upon substrata and FGF supplementation. Cytotechnology. 2014;**66**:27-35. DOI: 10.1007/s10616-012-9533-3

[91] Engebretson B, Mussett ZR, Sikavitsas VI. Tenocytic extract and mechanical stimulation in a tissue-engineered tendon construct increases cellular proliferation and ECM deposition. Biotechnology Journal. DOI: 10.1002/biot.201600595

[92] Veronesi F, Torricelli P, Della Bella E, Pagani S, Fini M. In vitro mutual interaction between tenocytes and adipose-derived mesenchymal stromal cells. Cytotherapy. 2015;**17**:215-223. DOI: 10.1016/j.jcyt.2014.10.006

[93] Zarychta-Wiśniewska W, Burdzinska A, Kulesza A, Gala K, Kaleta B, Zielniok K, et al. Bmp-12 activates tenogenic pathway in human adipose stem cells and affects their immunomodulatory and secretory properties. BMC Cell Biology. 2017;**18**:13. DOI: 10.1186/s12860-017-0129-9

[94] Wu T, Liu Y, Wang B, Sun Y, Lee WYW, Xu J, et al. The use of co-cultured mesenchymal stem cells with tendon-derived stem cells as a better cell source for tendon repair. Tissue Engineering Parts A. DOI: 10.1089/ten.TEA.2016.0248

[95] Taylor SE, Vaughan-Thomas A, Clements DN, Pinchbeck G, Macrory LC, Smith RK, et al. Gene expression markers of tendon fibroblasts in normal and diseased tissue compared to monolayer and three dimensional culture systems. BMC Musculoskeletal Disorders. 2009;**10**:27

[96] Clements LE, Garvican ER, Dudhia J, Smith RKW. Modulation of mesenchymal stem cell genotype and phenotype by extracellular matrix proteins. Connective Tissue Research. 2016;**57**:443-453. DOI: 10.1080/03008207.2016.1215442

[97] Garvican ER, Dudhia J, Alves AL, Clements LE, Plessis FD, Smith RK. Mesenchymal stem cells modulate release of matrix proteins from tendon surfaces in vitro: A potential beneficial therapeutic effect. Regenerative Medicine. 2014;**9**:295-308. DOI: 10.2217/rme.14.7

[98] Manning CN, Martel C, Sakiyama-Elbert SE, Silva MJ, Shah S, Gelberman RH, et al. Adipose-derived mesenchymal stromal cells modulate tendon fibroblast responses to macrophage-induced inflammation in vitro. Stem Cell Research & Therapy. 2015;**6**:74. DOI: 10.1186/s13287-015-0059-4

[99] Veronesi F, Della Bella E, Torricelli P, Pagani S, Fini M. Effect of adipose-derived mesenchymal stromal cells on tendon healing in aging and estrogen deficiency: An in vitro co-culture

model. Cytotherapy. 2015;**17**:1536-1544. DOI: 10.1016/j.jcyt.2015.07.007

[100] Ekwueme EC, Shah JV, Mohiuddin M, Ghebes CA, Crispim JF, Saris DBF, et al. Cross-talk between human tenocytes and bone marrow stromal cells potentiates extracellular matrix Remodeling In vitro. Journal of Cellular Biochemistry. 2016;**117**:684-693. DOI: 10.1002/jcb.25353

[101] Lange-Consiglio A, Perrini C, Tasquier R, Deregibus MC, Camussi G, Pascucci L, et al. Equine amniotic microvesicles and their anti-inflammatory potential in a tenocyte model in vitro. Stem Cells and Development. 2016;**25**:610-621. DOI: 10.1089/scd.2015.0348

[102] Long C, Wang Z, Legrand A, Chattopadhyay A, Chang J, Fox PM. Tendon tissue engineering: Mechanism and effects of human tenocyte coculture with adipose-derived stem cells. The Journal of Hand Surgery. 2018;**43**:183.e1-183.e9. DOI: 10.1016/j.jhsa.2017.07.031

[103] Chen Q, Liang Q, Zhuang W, Zhou J, Zhang B, Xu P, et al. Tenocyte proliferation and migration promoted by rat bone marrow mesenchymal stem cell-derived conditioned medium. Biotechnology Letters. 2018;**40**:215-224. DOI: 10.1007/s10529-017-2446-7

[104] Costa-Almeida R, Berdecka D, Rodrigues MT, Reis RL, Gomes ME. Tendon explant cultures to study the communication between adipose stem cells and native tendon niche. Journal of Cellular Biochemistry. 2018;**119**:3653-3662. DOI: 10.1002/jcb.26573

[105] Costa-Almeida R, Calejo I, Reis RL, Gomes ME. Crosstalk between adipose stem cells and tendon cells reveals a temporal regulation of tenogenesis by matrix deposition and remodeling. Journal of Cellular Physiology. 2018;**233**:5383-5395. DOI: 10.1002/jcp.26363

[106] Machova Urdzikova L, Sedlacek R, Suchy T, Amemori T, Ruzicka J, Lesny P, et al. Human multipotent mesenchymal stem cells improve healing after collagenase tendon injury in the rat. Biomedical Engineering Online. 2014;**13**:42. DOI: 10.1186/1475-925X-13-42

[107] Selek O, Buluç L, Muezzinoğlu B, Ergün RE, Ayhan S, Karaöz E. Mesenchymal stem cell application improves tendon healing via anti-apoptotic effect (animal study). Acta Orthopaedica et Traumatologica Turcica. 2014;**48**:187-195. DOI: 10.3944/AOTT.2014.2985

[108] Chen H-S, Su Y-T, Chan T-M, Su Y-J, Syu W-S, Harn H-J, et al. Human adipose-derived stem cells accelerate the restoration of tensile strength of tendon and alleviate the progression of rotator cuff injury in a rat model. Cell Transplantation. 2015;**24**:509-520. DOI: 10.3727/096368915X686968

[109] Al-Ani MK, Xu K, Sun Y, Pan L, Xu Z, Yang L. Study of bone marrow mesenchymal and tendon-derived stem cells transplantation on the regenerating effect of achilles tendon ruptures in rats. Stem Cells International. 2015;**2015**:984146. DOI: 10.1155/2015/984146

[110] Oshita T, Tobita M, Tajima S, Mizuno H. Adipose-derived stem cells improve collagenase-induced tendinopathy in a rat model. The American Journal of Sports Medicine. 2016;**44**:1983-1989. DOI: 10.1177/0363546516640750

[111] Yuksel S, Guleç MA, Gultekin MZ, Adanır O, Caglar A, Beytemur O, et al. Comparison of the early period effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on the Achilles tendon

ruptures in rats. Connective Tissue Research. 2016;**57**:360-373. DOI: 10.1080/03008207.2016.1189909

[112] de Aro AA, Carneiro GD, Teodoro LFR, da Veiga FC, Ferrucci DL, Simões GF, et al. Injured achilles tendons treated with adipose-derived stem cells transplantation and GDF-5. Cell. DOI: 10.3390/cells7090127

[113] Adams SB Jr, Thorpe MA, Parks BG, Aghazarian G, Allen E, Schon LC. Stem cell-bearing suture improves Achilles tendon healing in a rat model. Foot & Ankle International. 2014;**35**:293-299. DOI: 10.1177/1071100713519078

[114] Lee SY, Kwon B, Lee K, Son YH, Chung SG. Therapeutic mechanisms of human adipose-derived mesenchymal stem cells in a rat tendon injury model. The American Journal of Sports Medicine. 2017;**45**:1429-1439. DOI: 10.1177/0363546517689874

[115] Peach MS, Ramos DM, James R, Morozowich NL, Mazzocca AD, Doty SB, et al. Engineered stem cell niche matrices for rotator cuff tendon regenerative engineering. PLoS One. 2017;**12**:e0174789. DOI: 10.1371/journal.pone.0174789

[116] Otabe K, Nakahara H, Hasegawa A, Matsukawa T, Ayabe F, Onizuka N, et al. Transcription factor Mohawk controls tenogenic differentiation of bone marrow mesenchymal stem cells in vitro and in vivo. Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society. 2015;**33**:1-8. DOI: 10.1002/jor.22750

[117] Chiou GJ, Crowe C, McGoldrick R, Hui K, Pham H, Chang J. Optimization of an injectable tendon hydrogel: The effects of platelet-rich plasma and adipose-derived stem cells on tendon healing in vivo. Tissue Engineering Parts A. 2015;**21**:1579-1586. DOI: 10.1089/ten.TEA.2014.0490

[118] Aktas E, Chamberlain CS, Saether EE, Duenwald-Kuehl SE, Kondratko-Mittnacht J, Stitgen M, et al. Immune modulation with primed mesenchymal stem cells delivered via biodegradable scaffold to repair an Achilles tendon segmental defect. Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society. 2017;**35**:269-280. DOI: 10.1002/jor.23258

[119] Behfar M, Javanmardi S, Sarrafzadeh-Rezaei F. Comparative study on functional effects of allotransplantation of bone marrow stromal cells and adipose derived stromal vascular fraction on tendon repair: A biomechanical study in rabbits. Cell Journal. 2014;**16**:263-270

[120] He M, Gan AWT, Lim AYT, Goh JCH, Hui JHP, Chong AKS. Bone marrow derived mesenchymal stem cell augmentation of rabbit flexor tendon healing. Hand Surgery: An International Journal Devoted to Hand and upper limb surgery and related research : journal of the Asia-Pacific Federation of Societies for Surgery of the Hand. 2015;**20**:421-429. DOI: 10.1142/S0218810415500343

[121] Deng D, Wang W, Wang B, Zhang P, Zhou G, Zhang WJ, et al. Repair of Achilles tendon defect with autologous ASCs engineered tendon in a rabbit model. Biomaterials. 2014;**35**:8801-8809. DOI: 10.1016/j.biomaterials.2014.06.058

[122] Cai J, Yang Y, Ai C, Jin W, Sheng D, Chen J, et al. Bone marrow stem cells-seeded polyethylene terephthalate scaffold in repair and regeneration of rabbit achilles tendon. Artificial Organs. DOI: 10.1111/aor.13298

[123] Gelberman RH, Linderman SW, Jayaram R, Dikina AD, Sakiyama-Elbert S, Alsberg E, et al. Combined administration of ASCs and BMP-12 promotes an m2 macrophage phenotype and enhances tendon healing. Clinical

Orthopaedics and Related Research. 2017;**475**:2318-2331. DOI: 10.1007/s11999-017-5369-7

[124] Gelberman RH, Shen H, Kormpakis I, Rothrauff B, Yang G, Tuan RS, et al. Effect of adipose-derived stromal cells and BMP12 on intrasynovial tendon repair: A biomechanical, biochemical, and proteomics study. Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society. 2016;**34**:630-640. DOI: 10.1002/jor.23064

[125] Shen H, Jayaram R, Yoneda S, Linderman SW, Sakiyama-Elbert SE, Xia Y, et al. The effect of adipose-derived stem cell sheets and CTGF on early flexor tendon healing in a canine model. Scientific Reports. 2018;**8**:11078. DOI: 10.1038/s41598-018-29474-8

[126] Shen H, Kormpakis I, Havlioglu N, Linderman SW, Sakiyama-Elbert SE, Erickson IE, et al. The effect of mesenchymal stromal cell sheets on the inflammatory stage of flexor tendon healing. Stem Cell Research & Therapy. 2016;**7**:144. DOI: 10.1186/s13287-016-0406-0

[127] Khan MR, Dudhia J, David FH, de Godoy R, Mehra V, Hughes G, et al. Bone marrow mesenchymal stem cells do not enhance intra-synovial tendon healing despite engraftment and homing to niches within the synovium. Stem Cell Research & Therapy. 2018;**9**:169. DOI: 10.1186/s13287-018-0900-7

[128] Scharf A, Holmes S, Thoresen M, Mumaw J, Stumpf A, Peroni J. Superparamagnetic iron oxide nanoparticles as a means to track mesenchymal stem cells in a large animal model of tendon injury. Contrast Media & Molecular Imaging. 2015;**10**:388-397. DOI: 10.1002/cmmi.1642

[129] Lacitignola L, Staffieri F, Rossi G, Francioso E, Crovace A. Survival of bone marrow mesenchymal stem cells labelled with red fluorescent protein in an ovine model of collagenase-induced tendinitis. Veterinary and Comparative Orthopaedics and Traumatology. 2014;**27**:204-209. DOI: 10.3415/VCOT-13-09-0113

[130] Brandão JS, Alvarenga ML, Pfeifer JPH, Dos Santos VH, Fonseca-Alves CE, Rodrigues M, et al. Allogeneic mesenchymal stem cell transplantation in healthy equine superficial digital flexor tendon: A study of the local inflammatory response. Research in Veterinary Science. 2018;**118**:423-430. DOI: 10.1016/j.rvsc.2018.03.012

[131] Conze P, van Schie HT, van WR, Staszyk C, Conrad S, Skutella T, et al. Effect of autologous adipose tissue-derived mesenchymal stem cells on neovascularization of artificial equine tendon lesions. Regenerative Medicine. 2014;**9**:743-757. DOI: 10.2217/rme.14.55

[132] Geburek F, Mundle K, Conrad S, Hellige M, Walliser U, van Schie HT, et al. Tracking of autologous adipose tissue-derived mesenchymal stromal cells with in vivo magnetic resonance imaging and histology after intralesional treatment of artificial equine tendon lesions–A pilot study. Stem Cell Research & Therapy. 2016;**7**:21. DOI: 10.1186/s13287-016-0281-8

[133] Geburek F, Roggel F, van Schie HTM, Beineke A, Estrada R, Weber K, et al. Effect of single intralesional treatment of surgically induced equine superficial digital flexor tendon core lesions with adipose-derived mesenchymal stromal cells: A controlled experimental trial. Stem Cell Research & Therapy. 2017;**8**:129. DOI: 10.1186/s13287-017-0564-8

[134] Becerra P, Valdes Vazquez MA, Dudhia J, Fiske-Jackson AR, Neves F, Hartman NG, et al. Distribution of injected technetium(99m)-labeled mesenchymal stem cells in horses with

naturally occurring tendinopathy. Journal of Orthopaedic Research. 2013;**31**:1096-1102. DOI: 10.1002/jor.22338

[135] Sole A, Spriet M, Padgett KA, Vaughan B, Galuppo LD, Borjesson DL, et al. Distribution and persistence of technetium-99 hexamethyl propylene amine oxime-labelled bone marrow-derived mesenchymal stem cells in experimentally induced tendon lesions after intratendinous injection and regional perfusion of the equine distal limb. Equine Veterinary Journal. 2013;**45**:726-731. DOI: 10.1111/evj.12063

[136] Carvalho AM, Yamada AL, Golim MA, Alvarez LE, Hussni CA, Alves AL. Evaluation of mesenchymal stem cell migration after equine tendonitis therapy. Equine Veterinary Journal. 2014;**46**:635-638. DOI: 10.1111/evj.12173

[137] Ahrberg AB, Horstmeier C, Berner D, Brehm W, Gittel C, Hillmann A, et al. Effects of mesenchymal stromal cells versus serum on tendon healing in a controlled experimental trial in an equine model. BMC Musculoskeletal Disorders. 2018;**19**:230. DOI: 10.1186/s12891-018-2163-y

[138] Berner D, Brehm W, Gerlach K, Gittel C, Offhaus J, Paebst F, et al. Longitudinal cell tracking and simultaneous monitoring of tissue regeneration after cell treatment of natural tendon disease by low-field magnetic resonance imaging. Stem Cells International. 2016;**2016**:1207190. DOI: 10.1155/2016/1207190

[139] Burk J, Berner D, Brehm W, Hillmann A, Horstmeier C, Josten C, et al. Long-term cell tracking following local injection of mesenchymal stromal cells in the equine model of induced tendon disease. Cell Transplantation. 2016;**25**:2199-2211. DOI: 10.3727/096368916X692104

[140] Romero A, Barrachina L, Ranera B, Remacha AR, Moreno B, de Blas I, et al. Comparison of autologous bone marrow and adipose tissue derived mesenchymal stem cells, and platelet rich plasma, for treating surgically induced lesions of the equine superficial digital flexor tendon. Veterinary Journal (London, England : 1997). 2017;**224**:76-84. DOI: 10.1016/j.tvjl.2017.04.005

[141] Smith RK, Werling NJ, Dakin SG, Alam R, Goodship AE, Dudhia J. Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. PLoS One. 2013;**8**:e75697. DOI: 10.1371/journal.pone.0075697

[142] Le Blanc K. Immunomodulatory effects of fetal and adult mesenchymal stem cells. Cytotherapy. 2003;**5**:485-489. DOI: 10.1080/14653240310003611

[143] Peffers MJ, Collins J, Loughlin J, Proctor C, Clegg PD. A proteomic analysis of chondrogenic, osteogenic and tenogenic constructs from ageing mesenchymal stem cells. Stem Cell Research & Therapy. 2016;**7**:133. DOI: 10.1186/s13287-016-0384-2

[144] Hillmann A, Ahrberg AB, Brehm W, Heller S, Josten C, Paebst F, et al. Comparative characterization of human and equine mesenchymal stromal cells: A basis for translational studies in the equine model. Cell Transplantation. 2016;**25**(1):109-124. DOI: 10.3727/096368915X687822

[145] Phelps J, Sanati-Nezhad A, Ungrin M, Duncan NA, Sen A. Bioprocessing of mesenchymal stem cells and their derivatives: Toward cell-free therapeutics. Stem Cells International. 2018;**2018**:9415367. DOI: 10.1155/2018/9415367

[146] Liu Y, Suen C-W, Zhang J-F, Li G. Current concepts on tenogenic differentiation and clinical applications. Journal of Orthopaedic Translation. 2017;**9**:28-42. DOI: 10.1016/j.jot.2017.02.005

[147] Zhang Y-J, Chen X, Li G, Chan K-M, Heng BC, Yin Z, et al. Concise review: Stem cell fate guided by bioactive molecules for tendon regeneration. Stem Cells Translational Medicine. 2018;**7**:404-414. DOI: 10.1002/sctm.17-0206

[148] Kuo CK, Tuan RS. Mechanoactive tenogenic differentiation of human mesenchymal stem cells. Tissue Engineering. Part A. 2008;**14**:1615-1627

[149] Butler DL, Juncosa-Melvin N, Boivin GP, Galloway MT, Shearn JT, Gooch C, et al. Functional tissue engineering for tendon repair: A multidisciplinary strategy using mesenchymal stem cells, bioscaffolds, and mechanical stimulation. Journal of Orthopaedic Research. 2008;**26**:1-9

[150] Nirmalanandhan VS, Dressler MR, Shearn JT, Juncosa-Melvin N, Rao M, Gooch C, et al. Mechanical stimulation of tissue engineered tendon constructs: Effect of scaffold materials. Journal of Biomechanical Engineering. 2007;**129**:919-923

[151] Agata H, Watanabe N, Ishii Y, Kubo N, Ohshima S, Yamazaki M, et al. Feasibility and efficacy of bone tissue engineering using human bone marrow stromal cells cultivated in serum-free conditions. Biochemical and Biophysical Research Communications. 2009;**382**:353-358. DOI: 10.1016/j.bbrc.2009.03.023

[152] Alberton P, Popov C, Pragert M, Kohler J, Shukunami C, Schieker M, et al. Conversion of human bone marrow-derived mesenchymal stem cells into tendon progenitor cells by ectopic expression of scleraxis. Stem Cells and Development. 2012;**21**: 846-858. DOI: 10.1089/scd.2011.0150

[153] Chen YJ, Huang CH, Lee IC, Lee YT, Chen MH, Young TH. Effects of cyclic mechanical stretching on the mRNA expression of tendon/ligament-related and osteoblast-specific genes in human mesenchymal stem cells. Connective Tissue Research. 2008;**49**:7-14

[154] Xu B, Song G, Ju Y, Li X, Song Y, Watanabe S. RhoA/ROCK, cytoskeletal dynamics, and focal adhesion kinase are required for mechanical stretch-induced tenogenic differentiation of human mesenchymal stem cells. Journal of Cellular Physiology. 2012;**227**:2722-2729. DOI: 10.1002/jcp.23016

[155] Maharam E, Yaport M, Villanueva NL, Akinyibi T, Laudier D, He Z, et al. Rho/Rock signal transduction pathway is required for MSC tenogenic differentiation. Bone Research. 2015;**3**:15015. DOI: 10.1038/boneres.2015.15

[156] Chen S, Deng G, Li K, Zheng H, Wang G, Yu B, et al. Interleukin-6 promotes proliferation but inhibits tenogenic differentiation via the Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT3) pathway in tendon-derived stem cells. Medical Science Monitor: International Medical Journal of experimental and Clinical Research. 2018;**24**:1567-1573

[157] Zhang K, Asai S, Yu B, Enomoto-Iwamoto M. IL-1beta irreversibly inhibits tenogenic differentiation and alters metabolism in injured tendon-derived progenitor cells in vitro. Biochemical and Biophysical Research Communications. 2015;**463**:667-672. DOI: 10.1016/j.bbrc.2015.05.122

[158] Liu C, Luo J-W, Liang T, Lin L-X, Luo Z-P, Zhuang Y-Q, et al. Matrix stiffness regulates the differentiation of tendon-derived stem cells through FAK-ERK1/2 activation. Experimental Cell Research. 2018;**373**(1-2):62-70. DOI: 10.1016/j.yexcr.2018.08.023

[159] Safi E, Ficklscherer A, Bondarava M, Betz O, Zhang A, Jansson V, et al. Migration of mesenchymal stem cells

of bursal tissue after rotator cuff repair in rats. Joints. 2018;**6**:4-9. DOI: 10.1055/s-0038-1636948

[160] Anasiz Y, Ozgul RK, Uckan-Cetinkaya D. A new chapter for mesenchymal stem cells: Decellularized extracellular matrices. Stem Cell Reviews. 2017;**13**:587-597. DOI: 10.1007/s12015-017-9757-x

[161] Riley GP, Harrall RL, Cawston TE, Hazleman BL, Mackie EJ. Tenascin-C and human tendon degeneration. The American Journal of Pathology. 1996;**149**:933-943

[162] Dunkman AA, Buckley MR, Mienaltowski MJ, Adams SM, Thomas SJ, Satchell L, et al. Decorin expression is important for age-related changes in tendon structure and mechanical properties. Matrix Biology. 2013;**32**:3-13. DOI: 10.1016/j.matbio.2012.11.005

[163] Yoon JH, Halper J. Tendon proteoglycans: Biochemistry and function. Journal of Musculoskeletal & Neuronal Interactions. 2005;**5**:22-34

[164] Popov C, Burggraf M, Kreja L, Ignatius A, Schieker M, Docheva D. Mechanical stimulation of human tendon stem/progenitor cells results in upregulation of matrix proteins, integrins and MMPs, and activation of p38 and ERK1/2 kinases. BMC Molecular Biology. 2015;**16**:6. DOI: 10.1186/s12867-015-0036-6

[165] Lozito TP, Tuan RS. Mesenchymal stem cells inhibit both endogenous and exogenous MMPs via secreted TIMPs. Journal of Cellular Physiology. 2011;**226**:385-396. DOI: 10.1002/jcp.22344

[166] Lozito TP, Jackson WM, Nesti LJ, Tuan RS. Human mesenchymal stem cells generate a distinct pericellular zone of MMP activities via binding of MMPs and secretion of high levels of TIMPs. Matrix Biology: Journal of the International Society for Matrix Biology. 2014;**34**:132-143. DOI: 10.1016/j.matbio.2013.10.003

[167] Eckhard U, Huesgen PF, Schilling O, Bellac CL, Butler GS, Cox JH, et al. Active site specificity profiling of the matrix metalloproteinase family: Proteomic identification of 4300 cleavage sites by nine MMPs explored with structural and synthetic peptide cleavage analyses. Matrix Biology: Journal of the International Society for Matrix Biology. 2016;**49**:37-60. DOI: 10.1016/j.matbio.2015.09.003

[168] Wang M, Yuan Q, Xie L. Mesenchymal stem cell-based immunomodulation: Properties and clinical application. Stem Cells International. 2018;**2018**:3057624. DOI: 10.1155/2018/3057624

[169] Midwood KS, Chiquet M, Tucker RP, Orend G. Tenascin-C at a glance. Journal of Cell Science. 2016;**129**:4321-4327. DOI: 10.1242/jcs.190546

[170] Tang C, Chen Y, Huang J, Zhao K, Chen X, Yin Z, et al. The roles of inflammatory mediators and immunocytes in tendinopathy. Journal of Orthopaedic Translation. 2018;**14**:23-33. DOI: 10.1016/j.jot.2018.03.003

[171] Lange-Consiglio A, Rossi D, Tassan S, Perego R, Cremonesi F, Parolini O. Conditioned medium from horse amniotic membrane-derived multipotent progenitor cells: Immunomodulatory activity in vitro and first clinical application in tendon and ligament injuries in vivo. Stem Cells and Development. 2013;**22**:3015-3024. DOI: 10.1089/scd.2013.0214

[172] Vallés G, Bensiamar F, Crespo L, Arruebo M, Vilaboa N, Saldaña L. Topographical cues regulate the crosstalk between MSCs and macrophages. Biomaterials. 2015;**37**:124-133. DOI: 10.1016/j.biomaterials.2014.10.028

[173] Cassano JM, Schnabel LV, Goodale MB, Fortier LA. Inflammatory licensed equine MSCs are chondroprotective and exhibit enhanced immunomodulation in an inflammatory environment. Stem Cell Research & Therapy. 2018;**9**:82. DOI: 10.1186/s13287-018-0840-2