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ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iebt20

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To cite this article: Filippo Migliorini, Markus Tingart & Nicola Maffulli (2020) Progress with stem cell therapies for tendon tissue regeneration, Expert Opinion on Biological Therapy, 20:11, 1373-1379, DOI: 10.1080/14712598.2020.1786532

To link to this article: https://doi.org/10.1080/14712598.2020.1786532

EXPERT OPINION ON BIOLOGICAL THERAPY
2020, VOL. 20, NO. 11, 1373–1379
https://doi.org/10.1080/14712598.2020.1786532

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REVIEW

Progress with stem cell therapies for tendon tissue regeneration

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ABSTRACT

Introduction: Chronic musculoskeletal pain is very prevalent, and accounts for major health-care expenses. Many of the present therapeutic modalities are only partially effective, and great interest is now posed on regenerative medicine.

Areas covered: The authors discuss the role of a variety of regenerative medicine options to induce and favor regeneration and healing of tendon tissue, focusing on the role of mesenchymal stem cell therapy and their derivatives.

Expert opinion: Stem cells, tissue engineering, and growth factors are new strategies for tendon repair and regeneration. MSCs not only can differentiate in tendon cells, but also secrete several cytokines that modulate inflammation and tissue healing. Future studies should be undertaken to overcome current obstacles to clinical translation. Further investigation of cell source, isolation, expansion, and differentiation methods, characterization of the tenogenic differentiation pathways, and clarifications of tendon-specific molecular markers are required. The role of donor variability, tendon type, and anatomic location also requires further understanding and research.

ARTICLE HISTORY
Received 2 May 2020
Accepted 19 June 2020

KEYWORDS
Regenerative medicine; tendon; tendinopathy; mesenchymal stem cells; tissue engineering

1. Introduction

Chronic musculoskeletal pain accounts for major financial burden worldwide: the increase in life expectancy has induced a greater rate of chronic musculoskeletal pain [1,2]. A new modality which is producing increasing interest in the management of chronic musculoskeletal pain is regenerative medicine [3,4,5]. In general, the process of healing follows three steps, regardless of the cause of damage: inflammation, proliferation, and remodeling [6,7]. These processes are characterized by complex biochemical interactions and signaling patterns between several cytokines, cells, and environment [8]. The remodeling stage results from the balance of regenerative and fibrotic processes [9]. This balance often does not result in a *restitutio ab integrum*, but rather in a fibrotic scar [9]. Mesenchymal stem cells (MSCs) have been proposed as possible treatment to shift the balance in favor of regeneration [5]. These cells can differentiate into any tissue, thus raising wide interests, broad researches, and applications in orthopedic surgery and musculoskeletal medicine [10,11]. MSC therapies are multidisciplinary, involving engineering, molecular biology, and medicine [12,13] (Table 1).

2. Clinical relevance

Tendon damages are common in orthopedic surgery, and in sports and musculoskeletal medicine [14]. The regeneration balance of tendon injuries is highly in favor of fibrotic healing [15,16]. From a biomechanical point of view, a healed tendon is not as efficient as an uninjured one [10,17]. The fibrotic scar that forms from the healing process compromises the biomechanical proprieties of the tendon, reducing its elasticity and promoting adherences [11,18]. Clinically, a higher risk of recurrence and/or development of chronic degenerative tendinopathies has been reported after tendon injuries [11,19,20]. Recovery time is often prolonged, thus considerably reducing recreational activities and quality of life [14,20]. Surgery is considered the ultimate intervention for tendinopathies [19,21]. However, up to 40% of the patients operated still experiences complications and functional limitations following tendon surgery [22,23]. The high rate of failure suggests that current surgical treatment is not sufficient and may not be appropriate, and further solutions are required. For chronic tendinopathy, local or systemic administration of anti-inflammatory agents, shock waves, physiotherapy, electromagnetic field stimulation, hyaluronic acid, platelet-rich plasma, or other growth factors have been employed [24,25,26]. However, these treatments have not proven effective for the definitive treatment of chronic tendinopathies [27].

Patients' BMI and comorbidities are recognized as risk factors for poor surgical outcome [28,29,30]. Female gender also represents a risk factor, with males reporting less symptoms, greater satisfaction, and better functional outcomes compared to females [31]. Age is also a well-known risk factor for poor outcomes after tendon repair surgery [32,33]. The elderly demonstrated impaired Achilles tendon healing after rupture,

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Article highlights

- The management of tendon healing is challenging and often leads tosuboptimal outcomes
- The regeneration balance of tendon injuries is highly in favor of fibrotic healing, compromising the biomechanical proprieties, elasticity, and promoting adherences
- Mesenchymal stem cells yield growing interest for tendon repair and regeneration.
- MSCs not only can differentiate in tendon cells, but also secrete several cytokines that modulate inflammation, enhancing a regenerative tissue healing.
- Future studies should be undertaken to overcome current obstacles to clinical translation: (1) cells source, isolation, expansion and differentiation methods, (2) characterization of the tenogenic differentiation pathways, (3) clarification of tendon-specific molecular markers and (4) the role of donor variability, tendon type, and anatomic location.

This box summarizes the key points contained in the article.

a two-fold risk of rotator cuff tears, and a three-fold risk to suffer a massive rotator cuff tear compared to the younger population [34,35]. In diabetes mellitus type II patients, hyperglycemia promotes collagen glycation and compromises the extracellular matrix (ECM) composition, with poor healing capabilities [36]. Hypercholesterolemia has also been associated with a higher risk of tendinopathy of the rotator cuff and Achilles tendons [37,38]. Smokers present thinner and harder tendons compared to nonsmokers, with increased risk of rupture and poor surgical outcomes [39,40]. Further, ovariectomized rats showed reduced Achilles tendon healing capability, evidencing that hormones may influence the healing processes in an animal model [41].

3. Tendon healing process

Hypovascularity and hypocellularity, along with minimal metabolic activity [42,43], may be related to the low healing capability of tendons, which involve both intrinsic and extrinsic cell populations [44]. Epitenon, endotenon, and tendon parenchyma cells are all involved in the intrinsic processes, while circulating cells or those from adjacent tissues pertain to the extrinsic ones [45]. However, the processes of tendon healing have not yet been fully elucidated. This may also be caused by the lack of optimal *in vitro* experimental models, a consequence of several reason. First, experimental models originate mainly from acute tendon section models [46]. Furthermore, there are considerable differences in species-related healing processes [47,48]. Indeed, probably there are no two species with the exactly same features of tendon healing [48]. Non-human primates, such the macaques, represent the gold-standard for tendon animal model, but are connected to high costs and ethical limitations [48]. Therefore, rats are widely used, but their tendon healing process is far different from the human one [48].

As all musculoskeletal structures, tendon healing runs into three main temporally and biochemically overlapping stages: inflammation, proliferation, remodeling [49,50]. The acute inflammation stage lasts up to three days. Acute tendon rupture provokes a bleeding that initiates inflammation (extrinsic pathway). Activated platelets release chemotactic and growth factors, which trigger the migration of inflammatory cells and activate the tenocytes [49,51]. Tenocytes are responsible for the synthesis of immature fibrous tissue, composed of fibronectin and Collagen (Col) type III [52]. Inflammatory cells secrete several cytokines, such as Insulin-Like Growth Factor 1 (IGF-1), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-Beta (TGF-β) (Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), which promote capillary permeability, chemoattraction, angiogenesis, cell migration, and stimulation and proliferation of macrophages and fibroblasts [10,53,55,56]. During the proliferation stage, intrinsic tenocytes migrate from the endotenon to the injury site, along with fibroblasts from the epitenon and the synovial sheath (intrinsic pathway). The latter, along with other smaller cells populations, is

Table 1. Clinical studies investigating MSC transplantation for tendon healing (VAS: Visual Analogic Scale; SPADI: shoulder pain and disability index; mMCPI: Mayo Clinic Performance Index; MRI: Magnetic Resonance Imaging; CS: Constant Score; Col I: Collagen Type 1; BM-MSCs: bone marrow-derived stem cells; AD-MSCs: adipose derived-mesenchymal stem cells).

Author, year	Study Design	Patients	Cells Source	Outcomes
Lee et al. 2015 [21]	Prospective trial	Six patients suffering from chronic epicondylitis	Autologous AD-MSCs injections	At last follow-up, VAS decreased by 52%, while mMCPI increased by 26.6%, and on ultrasound examination a reduction in defect areas was observed. No adverse effect was recorded.
Jo et al. 2018 [120]	Prospective trial	18 patients with partial-thickness rotator cuff	Autologous AD-MSCs injections	At last follow-up, the SPADI decreased by 80% and 77% in the mid- and high-dose groups, respectively. The high-dose group reported a reduction of the VAS by 71%. At ultrasonography, the defect area significantly decreased in almost all the patients. No adverse effect was recorded.
Havlas et al. 2015 [121]	Prospective trial	10 patients with rotator cuff tears	Autologous BM-MSCs Injections	At six months, the VAS scored 0/100 and all other scores were also improved. MRI scans showed fully healed and well-integrated tissue of the rotator cuff tendon attachment in all patients. No adverse effect was recorded.
Lamas et al. 2015 [122]	Randomized controlled trial	13 patients with full-thickness rotator cuff tears	Autologous BM-MSCs implantation	At 12 months, the CS improved by 31% in the BM-MSCs group. Three patients in the BM-MSCs group and one in the control group (only Col I) underwent revision surgery because of swelling, recurrent tear symptoms and reduced range of motion.
Lamas et al. [123]	Randomized controlled trial	13 patients with full-thickness rotator cuff tendon tears	Autologous BM-MSCs embedded in scaffold	The trial was interrupted because of the high recurrence rate. However, the authors reported better CS in favor of the BM-MSCs group, and similar imaging findings in the two groups.
Hurd et al. 2020 [124]	Randomized controlled trial	20 patients with partial-thickness rotator cuff tears	Autologous ADMSCs injections	AD-MSCs were safe, and lead to improved shoulder function without adverse effects at one-year follow-up

highly stimulated by immune cells, especially macrophages [51]. Cell migration, proliferation and phenotypical expression are stimulated by IGF-1, PDGF, TGF-β, and GDF [54,57,59,60]. This results in an early ECM composed mostly by fibronectin, proteoglycans, and Col III [18,61,62]. The remodeling phase starts approximately after two months and lasts up to two years, and is characterized by a decrease in cellularity in favor of fibrosis. Cytokines such as IGF-1, TGF-β, and GDF are secreted mostly by intrinsic cells and act to promote these changes [58,60,63,,64,65]. Tendon fibroblasts differentiate in myofibroblasts to contract the granulation tissue produced during the proliferation stage, thus transforming it in a finite fibrous scar [66,67] Col III is replaced by Col I, the fibers of which align along the direction of prevalent strain, to build stiffness and strength [58]. During this phase, there is a reduction in tendon vascularization and tenocyte metabolism [46].

4. Regenerative medicine to enhance tendon healing: stem cells

Stem cells have been defined as cells capable of long-term division and self-renewal, not committed, which can differentiate in all cell lineages [20,68]. Stem cells have excised wide interests, with broad researches and applications to treat musculoskeletal diseases [69,70,71,72]. Current evidence supports stem cell procedures for musculoskeletal disorders [73,74,75,76,77,78,79].

Stem cells are a population of non-committed cells able to differentiate into every cellular lineage; they have high proliferation potential, and can modulate the immune response and tissue tropism [80,81,82,83]. Stem cells have been hypothesized to promote regeneration in tendon healing process [84,85,86,87]. The goal of stem cell application is to modulate inflammation, organize ECM regeneration, and promote a tissue regeneration over scarring [7,88]. However, some differences in the various populations of stem cells must be pointed out.

Tendon stem/progenitor cells (TSPCs) are multipotent adult stem cells involved in healing process [89,90,91]. TSPCs have been reported to show clonogenicity, differentiation potential, and express specific stemness surface marks [92]. Moreover, these TSCs also express tenogenic markers, which make them a distinct stem cell population. The population of TSPCs reduces with aging, possibly accounting for the higher prevalence of tendinopathies in the elderly [92,93]. A recent study demonstrated that Bone Morphogenetic Proteins 12/13 (BMP12/13) in addiction with ascorbic acid activate the tenogenic differentiation of pluripotent stem cells *in vitro* [16]. The efficacy and feasibility of autologous tenocyte implantation are currently under clinical investigation (Phase 2–3 clinical trial, NCT01343836). TSPCs have been demonstrated to differentiate in tenocytes *in vitro* and in animal studies [94,95,96,97]. TSPCs account approximately for 4% of the tendon cellular population [98]: given this scarce number, *in vitro* expansion is required prior to injection to allow for therapeutic effects. This procedure is limited by the high risk of phenotype drift [99]. Recently, the use of epigenomic approaches has been proposed to address this issue (e.g. with inhibitors of histone deacetylase activity) and maintain a stable phenotype [100]. The epigenetic code is composed by highly complex biochemical mechanisms and pathways that control DNA accessibility (e.g. histone modifications, methylation, non-coding RNA) [101,102,103,104]. This control leads to an increased lineage commitment of the cell (differentiation), and can thus be used to limit phenotype drift during *ex vivo* expansion. Tenocytes have been demonstrated to express the thyroid hormone receptor [105]. The role of Thyroid-stimulating hormone (Tsh) has not been clearly defined. An overexpression of thyroid receptor isoforms is protective against tendon apoptosis and enhances proliferation in *in vitro* studies [106]. A further experimental study found that Triiodothyronine (T_3) combined ascorbic acid enhanced the tendon regeneration during the healing process, demonstrating close to the physiological orientation of fibers and capillarity, along with improved Col I/III ratio.

Several harvest sources of MSCs have been described for tendon healing: bone marrow (BM-MSCs), adipose tissue-derived (AD-MSCs), and other less common sites. Bone marrow cell population is composed by 0.01% to 0.001% of BM-MSCs [107], with a reduction in cell quantity and quality in the elderly [108]. These can be easily harvested via bone marrow aspiration (e.g. iliac crest). Further expansion and tenogenic differentiation can be obtained with several growth factors (e.g. Growth Differentiation Factors, GDF 5,6,7) [109,110]. The expression of tendon surface proteins (e.g. Tenomodulin) indicates tenogenic commitment [111,112]. This phase must be strictly controlled, as a longer expansion can induce an osteogenic lineage differentiation growth [108]. BM-MSCs secrete grow factors and other soluble cytokines that induce cellular proliferation and control tissue signaling [113] and enhance tenogenic proprieties of tendon resident cells [113]. Compared to BM-MSCs, AD-MSCs have higher availability, reduced donor-site morbidity, and higher cellular content. Similar to BM-MSCs, AD-MSCs enhance the tenogenic properties of tendon resident cells [114,115], and play a role in preserving the native tendon architecture, expediting ECM remodeling, and improving Col I/III ratio [115,116]. AD-MSCs are easier to differentiate in tenogenic cells and express more tenogenic genes (e.g. Tnmd, TcC, Dcn) as well as Col I and III [117]. These features make AD-MSCs more promising for tendon healing compared to BM-MSCs [14,118,119].

5. In-human applications of MScs for tendon repair

Several protocols for clinical trials investigating the role of MSCs for tendon healing have been currently registered, and investigations are ongoing (NCT03688308, NCT01788683, NCT02484950, NCT03449082, NCT03279796, NCT03752827, NCT03454737). The current literature lacks in definitive human clinical trials. Lee et al. [21] treated six patients suffering from chronic epicondylitis with allogenic AD-MSCs injections. Patients were followed at 0.5, 2, 6, 12, 26, 52 weeks. The visual analogic scale (VAS) and the modified Mayo Clinic Performance Index (mMCPI) were used as clinical scores, along with an ultrasound examination of the tendon defect area. At last follow-up, VAS decreased by 52%, while mMCPI increased by 26.6%, and on ultrasound examination, a reduction in defect areas was observed. No adverse effect was recorded. Jo et al. [120] treated

18 patients with partial-thickness rotator cuff tear with autologous AD-MSCs injections and reported data regarding safety and tolerability of dose escalations along with clinical data on disability (shoulder pain and disability index, SPADI). The SPADI decreased by 80% and 77% in the mid- and high-dose groups, respectively. The high-dose group reported a reduction of the VAS by 71%. At ultrasonography, the defect area significantly decreased in almost all the patients. No adverse effect was recorded. Another study evaluating autologous BM-MSCs infiltrations for rotator cuff tears was performed by Havlas et al. [121]. The authors prospectively analyzed eight patients at 3 and 6 months after the injection with the VAS, Constant Score (CS), and the University of California (UCLA) score. At six months, the VAS scored 0/100 and all other scores were also improved. MRI scans showed fully healed and well-integrated tissue of the rotator cuff tendon attachment in all patients. No adverse effect was recorded. Lamas et al. [122] performed a double-blind randomized placebo-controlled trial evaluating the safety and effectiveness of autologous MSCs implantation in patients with full-thickness rotator cuff tears. The study was performed on 13 patients: five patients were treated with a Col I implant, while eight patients received a Col I membrane combined with autologous BM-MSCs. At 12 months, the CS improved by 31% in the BM-MSCs group. The rate of tear and repair integrity was similar in both groups. Three patients in the BM-MSCs group and one in the control group underwent revision surgery because of swelling, recurrent tear symptoms, and reduced range of motion. Chronic synovitis with granulomatous tissue was histologically evidenced, and symptoms disappeared after revision surgery. Recently, the same author [123] compared the safety and efficacy of autologous BM-MSCs embedded in a xenogenic scaffold for full-thickness rotator cuff tendon tears in a randomized, double-blind placebo-controlled trial. Thirteen patients were enrolled: recurrence of the rupture occurred in five of eight patients of the BM-MSCs group, and in three of five patients in the control group. The trial was interrupted because of the high recurrence rate. However, the authors reported better CS in favor of the BM-MSCs group and similar imaging findings in the two groups. A recent RCT [124] compared the efficacy of autologous ADMSCs to corticosteroid injections in 20 patients with partial-thickness rotator cuff tears. ADMSCs were safe, and lead to improved shoulder function without adverse effects at 12-month follow-up.

6. Expert opinion

Acute tendon rupture and chronic tendinopathies are highly prevalent, and represent a consistent burden for health-care systems worldwide. Overall, even surgery does not result in full restoration of function, and many acute injuries evolve into chronic tendinopathies. Stem cells, tissue engineering and growth factors are gaining attentions in the scientific community to meet the demand for new strategies for tendon repair and regeneration. The role of MSCs is controversial and unclear. *In vitro* and *in vivo* investigations clearly identified MSCs among resident tendon cells, proving their involvement in regenerative processes of the tendon. MSCs not only can differentiate in tendon cells, but also secrete several cytokines that modulate inflammation and tissue healing. A deeper understanding of intrinsic and extrinsic biomechanical pathways and signaling, as well as of molecular mechanisms, will help identify the best type of uncommitted MSC for transplantation and boost the use of these cells in regenerative medicine. This synergic 'transplantation – potentiation' may offer new insights and prospective, reduce fibrosis, and improve regeneration. Future studies should be undertaken to overcome current obstacles to clinical translation. MSCs can be directly injected or can be reprocessed, purified, expanded, and then injected. This would lead to a more homogeneous population and higher concentration. However, these processes are controversial and no consensus has been reached. Further investigation of cell source, isolation, expansion, and differentiation methods, characterization of the tenogenic differentiation pathways, and clarifications of tendon-specific molecular markers are required. This reflects the limitations on tendon cell isolation and characterization. As the molecular markers to characterize tenocytes are still unclear, the definition of an exact lineage differentiation is not completely possible, thus considerably restricting the development of effective cell-based therapies. Initially, tendon cells were isolated following collagenase digestion in explanted tissues. In the past few years, several protocols for cell isolation have been developed, but no consensus has been reached. This process is further complicated by lack of molecular markers for the clear definition of tenocytes. Further, deeper understanding of the interactions between MSCs and tendon cells of their signaling pattern and influence on the regenerative cascade is required to develop appropriate therapeutic protocols. The role of donor variability, tendon type, and anatomic location also requires further understanding and research.

Funding

This paper is not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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