



REVIEW ARTICLE

Advances of stem cell based-therapeutic approaches for tendon repair



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Summary Tendon injuries are significant clinical problems. Current treatments often result in incomplete repair or healing, which may lead to reduced function and rupture. Stem cell-based therapy is a promising intervention for tendon repair. In this article, we attempt to provide a brief overview on the recent progress in the field, current understanding of the underlying mechanisms of the approach, and the potential of stem cell-based therapies beyond cell implantation. We conclude the review by sharing our viewpoints on the challenges, opportunities, and future directions of this approach.

The translational potential of this article: This paper reviews recent progress on stem cell-based therapeutic approaches for tendon repair, which highlights its translational potential and challenges.

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Introduction

Tendon injuries represent a common clinical problem that affects 30 million people annually [1]. The injuries can occur as a result of trauma, overuse, and aging, and are typically characterised by pain, inflammation, and

dysfunction [2–5]. Tendon injuries are currently managed by conservative treatment and surgical intervention [6]. Conservative treatments include injection corticosteroids, over-the-counter nonsteroidal anti-inflammatory drugs, physical therapy, and extracorporeal shock wave therapy [7]. However, tendon healing is usually slow and injured tendons may fail to regain their full function with only conservative treatment [8]. Surgical options are frequently considered for severe acute tendon injuries, such as rupture [9]. Surgical repair with grafts is the current standard treatment for tendon ruptures. There are significant

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limitations with this approach, which include continued pain and risk of re-rupture [10], tendon adhesions, formation of scar tissue, ectopic bone formation, and the lack of regeneration of fibrocartilage at the tendon-to-bone junction [8]. Furthermore, these conservative and surgical treatment approaches usually cannot completely restore tendon to their native composition, structure, and mechanical properties. Therefore, there is a critical need for more effective treatments.

The tendon is a connective tissue between muscle and bone [11] that is relatively acellular and avascular consisting of a hierarchical arrangement of collagen fibre bundles [12,13]. Tenocytes are the primary cell type within tendon, and they are responsible for the overall maintenance of tendon [11,14]. Tendon-resident stem cells, termed tendon-derived stem progenitor cells, (TSPCs), were identified by Bi et al. [15]. TSPCs are found in parallel collagen fibrils surrounded by the extracellular matrix, and have a specific niche, which includes extracellular matrix proteins biglycan and fibromodulin, that allows for tenocyte differentiation [15]. The function and fate of TSPCs may decline with age [16,17], under disease conditions (e.g. tendinopathy [18]), or as a result of injury [19].

Advances in stem cell-based therapeutic approaches have shown great potential for tissue repair and regeneration and may be a promising new intervention for tendon repair and regeneration. In this paper, we will primarily review the recent progress on stem cell implantation-based therapy, their therapeutic potential and underlying mechanisms. We will also discuss the potential of stem cell-based approaches beyond cell implantation, such as using stem cell-derived extracellular vesicles (EV) and enhancing endogenous stem homing. Finally, we will conclude the review by sharing our perspectives on the challenges, opportunities, and future directions for stem cell-based approaches for tendon repair and regeneration.

Stem cell-based approaches for tendon repair: an overview

Stem cells for tendon repair

Stem cells can be classified based on their lineage differential potential. Pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), can differentiate into any cell in the body. Multipotent stem cells, such as mesenchymal stem cells (MSCs), can develop into more than one cell type, but their differentiation potential is more limited compared to pluripotent cells.

ESCs represent a single source of cells that could be used to replace any other cell type in the body which is lost due to damage or disease, and therefore have a significantly broad potential in regenerative medicine, including the repair and regeneration of tendon [20]. Chen et al. demonstrated that human ESCs (hESCs), by differentiating into MSCs (hESC-MSCs) and incorporated into a fibrin gel [21] or onto a knitted silk-collagen sponge scaffold [22], and implanted into a tendon defect can improve tendon structural and mechanical properties in rats. Despite these promising results, whether this therapeutic strategy also

benefits patients with chronic tendon injuries such as tendinopathy clinically requires further investigation [23].

Induced pluripotent stem cells (iPSCs) are pluripotent stem cells that can be generated directly from adult cells by introducing four specific genes encoding transcription factors which convert adult somatic cells into pluripotent stem cells [23,24]. The implantation of cells differentiated from iPSCs have negligible immunogenicity [25], which is one reason why iPSCs have become attractive cell sources for tissue repair. Xu et al. [26] first reported iPSC-derived neural crest stem cells (NCSCs) suspended within a fibrin gel and implanted into a rat patellar tendon window defect significantly enhanced tendon healing, compared to that treated with fibrin gel alone. Interestingly, the implantation of the iPSC-NCSCs led to healed wound tissue that exhibited no ectopic bone or cartilage formation during the experimental study for 4 weeks [26]. iPSCs might be a potential source for stem cell-based therapy for tendon repair, but long-term outcome evaluation is critically required. Furthermore, a recent study using equine iPSCs *in vitro* indicated that although equine iPSCs expressed tendon-associated genes and proteins in two-dimensional differentiation assays, in contrast to equine embryonic stem cells, equine iPSCs failed to generate artificial tendons when cultured in three-dimensional collagen gels [27]. A thorough investigation of the regulatory mechanism that is critical for iPSCs to obtain the reparative and regenerative capacity for tendon repair is required.

MSCs bear the potential for differentiating into a variety of connective tissue cell types including tenocytes. MSCs can be derived from various tissue sources, such as bone marrow [(BM): BM-derived stem cells, (BMSCs)], tendon (TSPCs), and adipose tissue [adipose-derived stem cells, (ADSCs)]. Numerous animal studies have demonstrated that cell-based approaches using MSCs can improve tendon repair (reviewed in [28,29]).

Emerging cell sources for tendon repair include peripheral blood MSC, umbilical cord blood-mesenchymal stem cells (UCB-MSCs), and periodontal ligament cells. For example, injection of peripheral blood MSCs improved histologic features of a diseased tendon in a collagenase-induced tendinopathy model in sheep [30]. Allogeneic UCB-MSCs injected into naturally occurring tendinitis of the superficial digital flexor tendon led to higher performance and strength, as well as improved healing as assessed by ultrasound imaging [31]. Efficacy of UCB-MSCs in improving tendon-bone healing following anterior cruciate ligament reconstruction has also been demonstrated in a rabbit model [32]. Injection of UCB-MSCs into the interface between the bone tunnel and tendon graft improved the histologic appearance in the bone-tendon interface [32]. Furthermore, periodontal ligament-derived stem/progenitor cells, obtained from patients undergoing orthodontic treatment, improved healing of a full-thickness Achilles tendon defect, compared to an untreated defect, and resulted in similar efficacy compared to Achilles tendon-derived cells [33]. While many stem cell types have shown efficacy in animal models, a standard treatment protocol may be required to evaluate and compare the treatment outcome and identify the most promising stem cell option for a specific type of tendon repair.

Many studies only use one type of stem cell. Interestingly, combining multiple stem cells in one treatment led to

an enhanced therapeutic effect. Coculture of BM-MSCs and TSPCs in a 1:1 ratio promoted tenogenic differentiation with increased expression of tenogenic markers and collagen I [34]. Implantation of cell sheets formed using BM-MSCs and TSPCs into a rat patellar tendon window defect resulted in a significant improvement in tendon healing compared to defects treated with BM-MSCs or TSPCs alone [34].

While many stem cell types have been demonstrated to enhance tendon healing, cases with safety concerns and adverse effects have been reported. As shown in BMSCs [35] and ADSCs [36], there is risk of ectopic bone and tumour formation under special circumstances. In both ESCs and iPSCs, there is a risk of teratoma formation after transplantation [37]. Furthermore, obtaining stem cells such as TSPCs may lead to donor site morbidity. Therefore, obtaining a sufficient number of high quality stem cells for clinical application such as TSPCs is challenging. Particular attention should also be paid to stem cell culture conditions. For example, compared to TSPCs cultured under a normoxic condition (20% O₂), TSPCs cultured under hypoxic conditions (5% O₂) had higher cell proliferation and expression of stem cell markers Oct-4, Nanog, and SSEA-4 [38]. Moreover, TSPCs cultured under a hypoxic condition, seeded onto a decellularised tendon matrix and implanted subcutaneously in nude rats, led to the formation of more extensive tendon-like structures [38], suggesting that a hypoxic condition may be better suited for maintaining the stemness of TSPCs in culture.

Delivery and promotion of stem cell tenogenic potential

Appropriate delivery and the promotion of a stem cell's tenogenic potential during transplantation is critical for stem cell-based tendon repair and regeneration, which remains extremely challenging. Stem cell-based treatments are usually supplemented with other factors, such as pre-treating the cells with growth factors, modulating stem cell fate and lineage potential through genetic, epigenetic or physical means, combining treatment with bioactive molecules, or seeding stem cells on functional scaffolds.

Pretreating MSCs with growth factors, such as bone morphogenetic protein-12, to induce tenogenic differentiation prior to stem cell implantation, was reported to enhance repair of an Achilles defect in a rat model [39]. Similarly, a stepwise tenogenic differentiation approach of implanting MSCs that were first cultured with transforming growth factor (TGF)- β 1 for 7 days followed by connective tissue growth factor for another 7 days, led to better structural and mechanical properties in a rat patellar tendon repair model [40].

Gene transfer has also been used to overexpress certain transcription factors, such as scleraxis (Scx) [41], early growth response-1 (Egr1) [42], and Smad8 [43], to promote tenogenic differentiation before implantation. For example, the transplantation of Scx-transduced TSPCs promoted healing at the early stage of the tendon repair process in a rat patellar tendon injury model [41]. Injecting MSCs with enforced expression of Egr1 into the site of an Achilles tendon injury in rats increased the formation of

tendon-like tissues [42]. Furthermore, implanting MSCs overexpressing Smad8 into a full-thickness Achilles tendon defect in mice led to an improvement in the histologic appearance of the tendon and improved mechanical properties [43].

Together, these growth factor and gene transfer studies suggest that committing stem cells to differentiate towards the tendon lineage prior to implantation may be critical for successful tendon repair. As there is a concern of nontendinous tissue formation with stem cell implantation, it will be critical to extend the duration of these studies to determine whether these strategies could be used to avoid nontendinous tissue differentiation.

Biologics are often used as a supplemental treatment for stem cell-based approaches for tendon repair. For example, platelet-rich plasma (PRP) is an autologous source of various growth factors, including platelet-derived growth factor, TGF- β , insulin-like growth factor, fibroblastic growth factor, and vascular endothelial growth factor [44]. While clinical studies have not clearly demonstrated the efficacy of using PRP alone in enhancing tendon repair [45], the combination of PRP with stem cells may have an enhanced effect. BM-MSCs combined with PRP in fibrin glue, and wrapped around a hamstring tendon enhanced tendon-bone healing compared to PRP alone in a rabbit model of anterior cruciate ligament reconstruction [46]. In dogs with naturally-occurring supraspinatus tendinopathy, injection of ADSCs suspended in PRP into the diseased supraspinatus tendon led to improved pathology as assessed by ultrasound, and functional improvements in gait at 90 days following treatment [47]. While these animal studies suggest that PRP may enhance the reparative ability of stem cells for tendon repair, clinical use of this treatment strategy, to our knowledge, has not yet been reported.

Various scaffolds have been used in tendon repair to provide mechanical support and topographical cues to mimic the native tendon microenvironment, and to enhance tenogenic differentiation of stem cells. For example, aligned silk scaffolds have been demonstrated to induce tenogenic differentiation without overexpression of growth factors [48]. Implantation of TSPC-seeded knitted silk-collagen sponge scaffolds led to improved structural and biomechanical properties compared to controls [49]. In addition to the use of artificial scaffolds, cell sheets have also been experimentally used in tendon regeneration. The cell sheet is a thick layer of confluent cultured cells with its produced extracellular matrix. It has been demonstrated that sheets generated by these MSCs with enforced expression of transcription factor Mohawk (Mkx) promoted tendon repair in a mouse Achilles-tendon defect model [50]. It has also been shown that cell sheets consisting of connective tissue progenitors from hESC lines and foetal tissues surgically implanted into an Achilles tendon defect in nude mice resulted in improved histology and biomechanical properties compared to nontreated mice [51]. Stem cells also showed an enhanced therapeutic outcome when loaded onto a suture during surgery. In a rat Achilles tendon defect model, a suture loaded with MSCs resulted in higher ultimate failure strength compared to the suture-only group [52].

There has been a trend for combining multiple strategies for tendon regeneration. For example, hESCs-MSCs were engineered to overexpress *Scx* and seeded onto a silk-collagen sponge, prior to implantation in a rat Achilles tendon gap wound model. This three-factor combined treatment led to enhanced tendon repair, compared to injured tendons treated with hESC-MSCs seeded on the silk-collagen sponge [53]. ADSCs treated with a combination of a soluble extract of decellularised tendon extracellular matrix and TGF- β 3 treatment, and cultured on an aligned scaffold led to increased Col I matrix synthesis and improved organisation [54]. Together, these studies suggest that guiding MSCs towards tenogenic differentiation and providing a microenvironment that mimics the native tendon tissue are critical to success repair and regeneration of injured or diseased tendons.

Advances in understanding the mechanisms underlying stem cell-based implantation for tendon repair and regeneration

Significant progress has been made in understanding mechanisms underlying the observed therapeutic effect of stem cell-based treatments. For example, fluorescently labelled hESC-MSCs in a fibrin gel that were injected into a patellar wound site were detected 2 weeks after injection, but the number of cells present in the wound declined at 4 weeks [21]. The labelled cells exhibited a spindle-like shape and expressed tendon-specific genes, but not markers of pluripotency [21], suggesting that the injected stem cells differentiated towards the tenogenic lineage. The data further suggest that hESC-MSCs secreted human foetal tendon-specific matrix and differentiation factors at the injury site, which recruited host tendon progenitors that differentiated into tenocytes and produced collagen matrix [21].

In contrast to the hESC-MSC study, MSCs overexpressing *EGR1* (MSC-EGR1) injected into the site of tendon injury in rats were not detected 3 weeks after injection [42]. This suggests that the role of the implanted stem cells may be transient. The study further demonstrated that *EGR1* increased levels of *Tgfb2* mRNA and TGF- β 2 protein in rat tendons grafted with MSC-EGR1, suggesting a potential paracrine mechanism accounting for the therapeutic effects [42].

Consistently, a paracrine effect was also observed in iPSC-NCSCs transplanted into a rat patellar tendon injury [26]. The transplanted iPSC-NCSCs produced foetal tendon-related matrix proteins (collagen XIV, collagen III), stem cell recruitment-related factors (SDF-1, fractalkine), and tenogenic differentiation-related cytokines (e.g., bone morphogenetic protein-12, TGF- β 3, basic-fibroblastic growth factor), which together may accelerate the host endogenous repair process [26].

Potential stem cell-based approaches beyond stem cell implantation

Recent studies suggest soluble factors, especially extracellular vesicles (EV), released from stem cells may play a key role in tissue repair. EVs are small membrane vesicles that are secreted by most cells in culture. EVs were first

clearly described by Pan and Johnstone in 1983 [55]. EVs include microvesicles, exosomes, and apoptotic bodies. EVs differ both in their physicochemical parameters such as size, density, unique composition of lipids, proteins, and nucleic acids, and in their biogenesis. While microvesicles have a size ranging from 50 to 1000 nm [56], exosomes have diameters of 40–100 nm [57].

EVs, and in particular exosomes, carry various proteins, including those related to intracellular vesicle trafficking, cytoskeletal components, and signal transduction [58]. Exosomes also contain DNA [59], RNA, and microRNA [60]. In recent years, stem cell-derived exosomes have demonstrated the potential to treat many diseases and disorders, including cardiovascular ischaemia [61–63], kidney injury [64], liver fibrotic disease [65], and the healing of cutaneous wounds [66], and may bear potential for tendon repair and regeneration.

It has been demonstrated that stem cells have an innate homing ability due to cytokine receptors present on the cell [67]. Enhancing stem cells to their destination, in particular, to wounded or diseased tendon tissues using specific physical or chemical cues is an area worth intense investigation. It has been reported that delivery of CTGF into a full-thickness rupture of a rat patellar tendon increased the number of endogenous CD146⁺ stem/progenitor cells within the wound site, and led to a repaired tendon with histological features and structural properties similar to that in native patellar tendons [68].

Challenges, opportunities and future directions

Although significant progress has been made recently, clinical data regarding the therapeutic efficacy of using stem cells to treat tendon injuries and diseases is limited. A recent systematic review [69] identified four clinical trials [70–73] using BM- [71,73] and allogenic adipose-derived stem cells [70,72] for the treatment of tendon disorders (patellar tendinopathy, lateral epicondylar tendinopathy and rotator cuff tears). These four studies found that stem cell treatment led to improved tendon healing, as assessed by imaging, functional outcomes, and pain scores [70–73]. However, only one trial had a control group [71], and all four studies were not blinded, allowing for a high risk of biased results. Therefore, the results should be interpreted with caution [69]. Clearly, many basic and translational studies are needed before stem cell-based therapies can be recommended as a routine clinical treatment for tendon injuries and diseases.

Furthermore, many challenging questions still remain to be addressed, such as: (1) Which sources of stem cells are most promising or bear the best potential to be used? (2) Are there subpopulations of cells among the heterogenous population of MSCs that may bring a more favourable outcome? (3) What type of injuries or diseases require the implantation of stem cells? (4) Can biologics such as stem cell-derived exosomes replicate the therapeutic effect of stem cells in tendon injury and/or disease, and to which extent? Moreover, as there are many significant biological differences between acute or chronic tendon injuries, and the repair mechanisms following these injuries [74],

specific stem-cell therapy approaches may need to be tailored for each particular type of tendon injury. Research to address these questions and issues, may not only advance the basic understanding of the mechanisms underlying the roles stem cells play in tendon repair and regeneration but provide scientific merit and feasibility for stem cell-based approaches such as stem cell implantation, stem cell-derived biologics, and inducing endogenous stem homing. Finally, as a very critical step, it is important to establish standard methodologies and treatment protocols for harvesting, amplification, pretreatment, delivery, and postcell delivery treatment in preclinical studies and in clinical trials.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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