

# Stem cell technology for tendon regeneration: current status, challenges, and future research directions

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**Abstract:** Tendon injuries are a common cause of physical disability. They present a clinical challenge to orthopedic surgeons because injured tendons respond poorly to current treatments without tissue regeneration and the time required for rehabilitation is long. New treatment options are required. Stem cell-based therapies offer great potential to promote tendon regeneration due to their high proliferative, synthetic, and immunomodulatory activities as well as their potential to differentiate to the target cell types and undergo genetic modification. In this review, I first recapped the challenges of tendon repair by reviewing the anatomy of tendon. Next, I discussed the advantages and limitations of using different types of stem cells compared to terminally differentiated cells for tendon tissue engineering. The safety and efficacy of application of stem cells and their modified counterparts for tendon tissue engineering were then summarized after a systematic literature search in PubMed. The challenges and future research directions to enhance, optimize, and standardize stem cell-based therapies for augmenting tendon repair were then discussed.

**Keywords:** stem cells, tendon repair, tendon tissue engineering, tendon injuries

## Introduction

Tendon and ligament injuries are common clinical problems as a result of either overuse or aging. There are more than 30 million tendon and ligament injuries occurring annually worldwide.<sup>1</sup> These injuries often upset the balance between mobility and stability of the joint which results in abnormal loading that could damage other soft tissues in and around the joint that can progress into early onset of osteoarthritis, pain, disability, and eventually the need for joint replacement.<sup>2</sup> Their occurrence is particularly devastating to the elite athletes as it can be career-ending. The social and economic burden associated with these injuries presents a compelling argument to better understand their pathophysiology and develop appropriate treatments.

Tendon injury is currently managed by two approaches: 1) conservative treatment which aims to relieve pain and 2) surgical excision and repair. Irrespective of the approaches used, the treated tendon heals slowly and fails to regain its full function due to the formation of mechanically inferior scar tissue, ectopic bone, and adhesion or the lack of regeneration of fibrocartilage at the tendon to bone junction (TBJ). Repeated ruptures, joint stiffness, and restricted movement are common problems encountered even after repair.

The inability of tendon to self-repair and the inefficiency of current treatment regimens used clinically have sparked the exploration of alternative treatment strategies. The use of stem cells to repair tendon is particularly exciting and promising as stem cells

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have the potential to differentiate into tenocytes, show high proliferative and synthetic activities, and can secrete paracrine factors and exhibit immunomodulatory effects to promote tendon regeneration. However, a number of challenges have to be overcome before they can be used as a safe and effective therapeutic option for promoting tendon repair.

In this review, I aimed to present the recent advances, challenges, and future research directions of application of stem cells for tendon regeneration. I first recapped the anatomy of tendon. Then, I discussed the advantages and limitations of using different types of stem cells compared to terminally differentiated cells for tendon tissue engineering. Next, I summarized the literature regarding the safety and efficacy of application of stem cells and their modified counterparts for the promotion of tendon repair. Finally, I presented the challenges and future research directions to enhance, optimize, and standardize stem cell-based therapies for the augmentation of tendon repair.

## Why are tendons difficult to heal? A review of tendon anatomy

Tendon consists of collagen (mostly type I collagen) and elastin embedded in a proteoglycan-rich matrix. Collagen and elastin account for 65%–80% and 1%–2%, respectively, while proteoglycans account for 1%–5% of the tendon dry mass.<sup>3</sup> The tendon matrix is produced by tenoblasts and tenocytes that lie parallel between the longitudinally-arranged collagen fibers. The cellularity of tendon tissue is low (as opposed to epithelial tissue which has high cellularity), explaining the low turnover and poor self-healing capacity of the tissue. Recent studies have shown that tendon also contains resident stem cells which function to maintain tendon homeostasis during growth and repair.<sup>4,5</sup> Recent reports have also suggested that the change of tendon microenvironment after injury may induce erroneous differentiation of stem cells in tendon and cause pathological tendon ossification and failed tendon healing.<sup>6–8</sup>

The collagen molecules form cross-links and are packed in a quarter staggered fashion to form microfibrils, which are further aggregated together to form collagen fibrils. The staggering of collagen microfibrils and collagen fibrils produces the characteristic banding pattern of tendon under polarized microscopy. The collagen fibrils are grouped to form bigger units called collagen fibers.<sup>9</sup> The endotenon, which is a sheath of connective tissue, interacts with each collagen fiber and binds the fibers together. The collagen fibers are further organized into higher orders of primary (subfascicle), secondary (fascicle), and tertiary fiber bundles

to form the tendon. The entire tendon is surrounded by a thin connective tissue called epitenon. Some tendons (such as flexor tendon of fingers) are surrounded by a two-layer synovial sheath containing peritendinous fluid for lubrication as the tendons glide over the bone surfaces and prevention of bowstringing while some tendons (such as Achilles tendon) have the paratenon instead to reduce friction. The anatomy of tendon is illustrated in Figure 1.

Tendons contain blood vessels though it is considerably less than that in the other tissues such as muscles. The blood supply is particularly low in tendon regions that wrap around the bony pulleys.<sup>10</sup> Such areas of diminished or absent blood supply are commonly the sites of tendon degeneration and/or rupture, suggesting that blood supply is important for tendon repair after injury. Blood vessels enter tendon at the myotendinous junction, which then supply the rest of the tendon. They run parallel to the fascicles as well as within the endotenon. It is still controversial if entheses is an important site for the entry of blood vessels.<sup>11</sup>

The mid-substance of tendon is poorly innervated and the majority of nerve fibers are located within the tissue sheaths.<sup>12</sup> However, nerve fibers can grow into damaged or ruptured tendons in association with blood vessels. The nerve fibers may function to regulate blood flow within the tendon, collect sensory information (including pain), and relay this to the central nervous system.<sup>12</sup> The ingrowth of nerves into the injured tendon disappears as the tendon heals.<sup>13</sup> The tendon entheses is aneural and this may be associated with more frequent injuries at this region.<sup>14</sup>

Due to the relatively acellular and avascular nature of tendon as well as the change of tendon microenvironment after injury, tendon repair is slow and often incomplete.

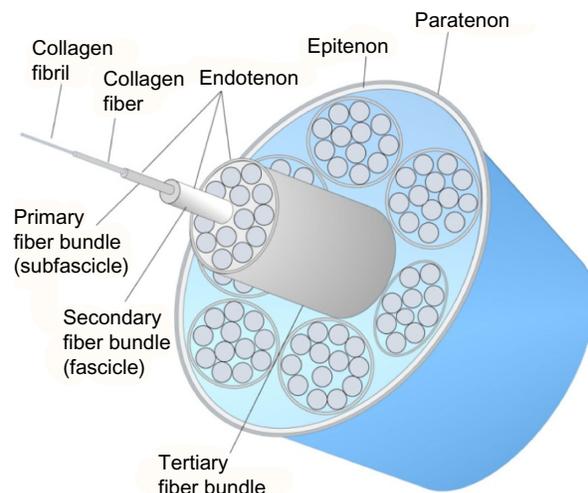


Figure 1 Anatomy of a tendon.

Tendons naturally heal with the formation of scar tissue and ectopic bone which makes them prone to failure.

## Stem cell-based tendon tissue engineering

Tissue engineering involves the use of cells, biomaterials, growth factors, enzymatic antagonists, or their combinations with the aim of directing a more sophisticated healing response to promote tissue repair. Both terminally differentiated cells and stem cells have been used in tendon tissue engineering. Among these different cell types, stem cells have attracted a great interest in tissue engineering as they can continuously reproduce themselves while maintaining the ability to differentiate into various cell types. The advantages and limitations of different cell types for tendon tissue engineering are discussed below.

### Advantages of stem cells compared to terminally differentiated cells for tendon tissue engineering

As tenocytes are the major cell type in tendon and are the key machinery driving tissue repair via the production of growth factors and synthesis of extracellular matrix, it is logical to use tenocytes as the cell source in tendon tissue engineering. There are three primary problems associated with the use of tenocytes for tendon tissue engineering. First, tenocytes are highly differentiated cells and have limited capacity to replicate and differentiate. The intrinsic healing ability of tendon fibroblasts is poor. Zhang and Wang have reported that tendon-derived stem cells (TDSCs) proliferated faster compared to tenocytes.<sup>15</sup> Second, phenotype drift and function loss are often observed during *in vitro* expansion of tenocytes.<sup>16,17</sup> The tenocytes became more rounded, the confluent cell density decreased, the ratio of type III to type I collagen increased while the expression of decorin decreased during *in vitro* cell passaging.<sup>16</sup> Tendon-selective genes including tenomodulin and thrombospondin 4 were rapidly downregulated in primary tendon fibroblasts cultured in monolayers and in organ cultures.<sup>18</sup> Third, tendons are relatively acellular and contain few tenocytes and there is the problem of donor site morbidity in autologous transplantation. As tenocytes are fibroblasts, some research groups use skin fibroblasts which are more easily accessible for tendon repair. However, skin fibroblasts are not specialized cells for maintaining the homeostasis of tendon. The first two problems associated with the use of differentiated cells remain. Both cell sources have a low risk of teratoma formation and hence have fewer safety concerns. There have been clinical trials reporting the

use of tenocytes<sup>19</sup> and skin fibroblasts<sup>20</sup> for the treatment of tendinopathy with promising results. Ultrasound-guided autologous tenocyte injection was reported to improve the clinical function and magnetic resonance imaging (MRI) tendinopathy scores in patients with refractory lateral epicondylitis at 4.5 years posttreatment.<sup>19</sup> On the other hand, ultrasound-guided injection of autologous skin fibroblasts was reported to improve the Victorian Institute of Sport Assessment score in patients with patellar tendinopathy at 6 months posttreatment.<sup>20</sup>

Embryonic stem cells (ESCs) have unlimited proliferative capacity and theoretically can be induced into all types of somatic cells for tissue regeneration. However, there is a risk of teratoma formation after transplantation.<sup>21</sup> Therefore, predifferentiation of ESCs to mesenchymal lineages is required prior to transplantation. As ESC-based therapies are particularly susceptible to generating tumors if the graft contains any undifferentiated cells, strategy to effectively remove the undifferentiated cells is needed.<sup>22</sup> There is also an ethical concern with the harvest of ESCs from embryos for tendon repair. The generation of induced pluripotent stem cells (iPSCs) from terminally differentiated cells eases the ethical concern of using ESCs. However, the risk of teratoma formation remains as iPSCs are pluripotent. Moreover, reprogramming factors associated with cell proliferation and tumorigenesis as well as integrated viral vectors are often used for the generation of iPSCs,<sup>23,24</sup> although there are now better and more efficient nonintegrating viral and nonviral vectors as well as small molecules for iPSC generation.<sup>24,25</sup> Similar to ESCs, predifferentiation of iPSCs to cells of mesenchymal lineages followed by separation of the differentiated cells from the residual pluripotent progeny are required prior to transplantation.<sup>23</sup> It is generally agreed that the differentiated cells derived from syngeneic iPSCs are not immunogenic and are not rejected after transplantation<sup>26,27</sup> although one earlier study has reported that undifferentiated iPSCs could induce immune response in the syngeneic recipient mice after transplantation.<sup>28</sup> Transplantation of ESC- or iPSC-derived tissues from an unrelated (allogeneic) donor that expresses foreign human leucocyte antigens may cause immunological rejection. Hence, the creation of stem cell banks comprising human leucocyte antigen-typed human ESCs and iPSCs is proposed to overcome the immunological barrier by providing human leucocyte antigen-matched (histocompatible) tissues for the target population.<sup>29</sup>

Unlike ESCs and iPSCs, mesenchymal stem cells (MSCs) have restricted self-renewal and lineage differentiation potential and hence have fewer safety concerns. The harvest of

MSCs is easy and does not raise an ethical issue when compared to ESCs. MSCs have higher proliferative and synthetic activities compared to terminally differentiated cells. For instances, bone marrow-derived stem cells (BMSCs) were reported to show higher collagen production and cell proliferation (as indicated by higher DNA content) after seeding on polylactide/glycolide suture material compared to anterior cruciate ligament and skin fibroblasts.<sup>30</sup> Similarly, others have reported that BMSCs possessed higher proliferation rate and collagen excretion in vitro as well as longer survival in the knee joint after transplantation when compared to anterior cruciate ligament or medial cruciate ligament fibroblasts.<sup>31</sup> BMSCs, but not tendon fibroblasts, were reported to stimulate biological and biomechanical healing of patellar tendon after injury.<sup>32</sup> MSCs are immune-privileged cells and hence allogeneic cells can be used for tendon repair,<sup>33–35</sup> although they also retain a degree of immunogenicity in some circumstances that may limit their longevity and attenuate their beneficial effects.<sup>36</sup> Recent studies have shown that MSCs had immunosuppressive effects. They were reported to switch the macrophage phenotype from pro-inflammatory to anti-inflammatory and secrete bioactive molecules that might promote tissue repair and disease treatment.<sup>37–40</sup> MSCs have been isolated from almost all types of tissues in the body. However, studies have shown variations in the stem cell properties of MSCs derived from different tissues.<sup>41</sup> Some MSC types therefore may perform better compared to the other types for tendon repair. Despite the encouraging findings of MSC transplantation for tendon repair, the transplantation of BMSCs into the rabbit tendon defect was reported to form ectopic bone in some tendon samples.<sup>42,43</sup> The transplanted BMSCs were found in the ectopic bone and expressed alkaline phosphatase, suggesting that some osteoblasts within the ectopic bone were derived from the implanted BMSCs.<sup>42,43</sup> While the authors have reported that the seeding density tested (1, 4, and 8 million cells/mL) did not affect the extent and frequency of ectopic bone formation after transplantation, they have speculated that the cell to collagen ratio and in vitro construct contraction might influence cell–cell contact and hence chances of ectopic bone formation after transplantation.<sup>43</sup> Tumor induction in mice after transplantation of undifferentiated BMSCs was reported when the cells were transplanted in scaffolds to syngeneic and immune-deficient recipients.<sup>44</sup> A recent study has shown that ESCs survived in greater numbers (constant number up to day 90 vs less than 5% at day 10) and migrated to longer distance compared to BMSCs after injection into damaged horse tendons.<sup>45</sup> Whether this difference in survival

rate and distribution affected the healing outcomes has not been reported. Further study is needed. Table 1 summarizes the advantages and limitations of different cell sources for tendon tissue engineering.

## Stem cells as vehicles for gene therapy and sustained delivery of bioactive factors

Although the clinical outcomes after stem cell transplantation were often encouraging, the mode of action of the transplanted cells remains unresolved. While it is generally assumed that stem cells promote tissue repair by direct engraftment and differentiation, differentiation of stem cells into target cell types is rarely shown. Furthermore, some studies have reported that only a small proportion of the cells persisted at the target sites and most of the cells were not detectable after 7–14 days posttransplantation.<sup>34,35</sup> There are evidences to suggest that stem cells may promote tissue repair via the secretion of paracrine factors that reduce inflammation and promote angiogenesis.<sup>34,35,37</sup> Microvesicles (100–1,000 nm) are membranous structures that arise from the budding of the plasma membrane.<sup>46</sup> A wide range of molecules including cytokines, growth factors as well as miRNA were identified in microvesicles derived from MSCs. Indeed, microvesicles were shown to promote the regeneration of a number of tissues (eg, lung, kidney, heart, liver, or nervous tissues) via transferring cytokines to the target or neighboring cells.<sup>47,48</sup> The production of microvesicles by stem cells may partially explain the paracrine effects observed in stem cell-based therapies. The use of cells that are either naturally or genetically engineered to provide a sustained delivery of multiple growth factors for tissue repair has an advantage over the application of single growth factor, which typically only has a half-life of minutes to hours. Stem cells therefore are useful as carriers of drugs and bioactive factors.<sup>49</sup>

## Current status of application of stem cells for tendon repair

The PubMed database was searched with the key words “stem cells tendon” on July 26–29, 2015 and “bone marrow stromal cells tendon” on August 17, 2015 with no restriction in language and year of publication. The studies were selected after reviewing the titles and abstracts. Original studies evaluating the safety and efficacy of stem cell for the promotion of TBJ repair of rotator cuff and Achilles tendon as well as tendon repair in animals and human were included. A total of 857 and 351 articles were identified with the key words, respectively. Of these, 112 studies are eligible for inclusion in this review. [Supplementary material](#)

**Table 1** Advantages and limitations of different cell sources for tendon tissue engineering

Pluripotency	Cell type	Advantages (common)	Advantages (specific)	Disadvantages (common)	Disadvantages (specific)
Terminally-differentiated cells	Skin fibroblast	<ul style="list-style-type: none"> <li>Low risk of teratoma formation</li> </ul>	<ul style="list-style-type: none"> <li>Easily available tissue source for cell isolation</li> <li>Shows fibroblast characteristics and produces collagen type I similar to tendon fibroblasts</li> </ul>	<ul style="list-style-type: none"> <li>Highly differentiated cells with limited proliferation rate and healing ability</li> <li>Phenotype drift and functional loss during in vitro expansion</li> </ul>	<ul style="list-style-type: none"> <li>Non-tenocytes that are not specialized for maintaining homeostasis of tendon and TBJ</li> </ul>
	Tendon fibroblast		<ul style="list-style-type: none"> <li>Major cell type in tendon responsible for the production of ECM in tendon and TBJ</li> </ul>		<ul style="list-style-type: none"> <li>Few tenocytes in tendon</li> <li>Donor site morbidity in autologous transplantation</li> </ul>
Pluripotent stem cells	ESC	<ul style="list-style-type: none"> <li>Unlimited proliferative capacity</li> <li>Pluripotent</li> </ul>		<ul style="list-style-type: none"> <li>Risk of teratoma formation</li> <li>May require predifferentiation to mesodermal lineage prior to transplantation</li> </ul>	<ul style="list-style-type: none"> <li>Ethical concern with the use of embryos for cell isolation</li> <li>Tissue source for cell isolation not easily available</li> </ul>
	iPSC		<ul style="list-style-type: none"> <li>Easily available tissue source for cell isolation</li> <li>No ethical concern for tissue source used for iPSC generation</li> </ul>	<ul style="list-style-type: none"> <li>Difficulty of removing the undifferentiated cells after in vitro stepwise differentiation</li> <li>Allogeneic transplantation may induce immunological rejection</li> </ul>	<ul style="list-style-type: none"> <li>Slow and inefficient reprogramming protocols</li> <li>Reprogramming factors and integrated viral vectors used for iPSC generation may be tumorigenic</li> </ul>
Multipotent stem cells	BMSC	<ul style="list-style-type: none"> <li>Less safety concern due to limited self-renewal potential and confined multilineage differentiation potential of cells of mesodermal lineage</li> <li>Higher proliferation rate and synthetic activity compared to fibroblasts</li> <li>Easily available tissue source for cell isolation</li> <li>No ethical concern for tissue source</li> <li>Immune-privileged cells suitable for allogeneic transplantation</li> <li>Immunomodulatory</li> </ul>		<ul style="list-style-type: none"> <li>Aging and loss of multilineage differentiation potential with in vitro cell passaging</li> </ul>	<ul style="list-style-type: none"> <li>Reported incidence of ectopic bone formation after transplantation</li> <li>Reported incidence of tumor induction when cells were transplanted in scaffolds to syngeneic and immune-deficient recipients</li> <li>Cell concentration decreases with volume of aspirated marrow due to dilution of bone marrow by peripheral blood</li> <li>Lower yield compared to other MSC sources</li> <li>Additional surgery and pain for cell isolation for autologous transplantation</li> </ul>
			ADSC	<ul style="list-style-type: none"> <li>Higher yield, clonogenicity, proliferation rate, and some lineage differentiation potential compared to BMSCs</li> </ul>	
	TDSC		<ul style="list-style-type: none"> <li>Likely imprinted to produce tendon and junctional tissues under the influence of local environmental niche</li> </ul>		<ul style="list-style-type: none"> <li>Donor site morbidity and hence not practical for autologous transplantation</li> </ul>

(Continued)

Table 1 (Continued)

Pluripotency	Cell type	Advantages	Disadvantages
	AMSC	<ul style="list-style-type: none"> <li>Higher yield, clonogenicity, proliferation rate, and some lineage differentiation potential compared to BMSCs</li> <li>Higher proliferation rate compared to BMSCs</li> <li>Non-invasive and no problem of donor site morbidity</li> </ul>	<ul style="list-style-type: none"> <li>Can only be isolated from mothers/newborns</li> <li>Limited quantities from each donor</li> <li>Lower success rate of isolation compared to other MSC sources</li> </ul>
	UCB-MSC	<ul style="list-style-type: none"> <li>Higher proliferation rate compared to BMSCs</li> <li>Non-invasive and no problem of donor site morbidity</li> </ul>	<ul style="list-style-type: none"> <li>Can only be isolated from mothers/newborns</li> <li>Limited quantities from each donor</li> <li>Lower success rate of isolation compared to other MSC sources</li> </ul>

**Abbreviations:** TBJ, tendon to bone junction; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; BMSC, bone marrow-derived stem cell; ADSC, adipose-derived stem cell; TDSC, tendon-derived stem cell; AMSC, amniotic tissue-derived mesenchymal stem cell; UCB-MSC, umbilical cord blood-mesenchymal stem cell; ECM, extracellular matrix; MSC, mesenchymal stem cells.

summarizes the preclinical and clinical studies (rows in gray color) on the application of stem cells for the promotion of tendon repair.

BMSCs, blood-derived MSCs, adipose-derived stem cells (ADSCs), TDSCs, ESCs, umbilical cord blood-MSCs, amniotic MSCs, ESCs, and iPSCs were the commonly used stem cell types for tendon repair. Ninety-nine studies are animal studies and five are clinical trials (Supplementary material). Achilles tendon transection or segmental tendon defects, patellar tendon window injury, collagenase-induced Achilles or superficial digital flexor tendon injury, naturally-occurring superficial digital flexor tendon injury and TBJ injury at the Achilles, infraspinatus or supraspinatus tendons were the commonly used tendon injury models. Most of the animal studies were based on small animal models such as rats and rabbits. The large animal models were collagenase-induced or naturally-occurring tendon injury in sheep or horses and most of them were on horses. Injury of the superficial digital flexor tendon is very common in horses and this explains why it is often used as a model of tendinopathy. Instead of injecting or transplanting stem cells, one study injected the conditioned medium of amniotic membrane-derived mesenchymal progenitor cells to promote healing of naturally-occurring tendon injuries in horses and reported a significant lower rate of reinjury compared to the untreated animals.<sup>40</sup> Except ten studies, the other preclinical studies reported the benefits of transplanting stem cells on tendon repair (Supplementary material). All the large animal studies have shown some positive effects of stem cell transplantation on tendon repair although there were variations in the quality, outcome assessments, and follow-up duration of the studies. While Gulotta et al,<sup>50</sup> Valencia Mora et al,<sup>51</sup> and Barco et al<sup>52</sup> have reported that the transplantation of BMSCs did not improve supraspinatus TBJ healing in rat models, transosseous drilling of the greater tuberosity to release BMSCs to the suture zone was shown to improve supraspinatus TBJ repair.<sup>53</sup> The transplantation of BMSCs was reported to improve Achilles TBJ<sup>54</sup> and infraspinatus TBJ<sup>55</sup> repair whereas the transplantation of ADSCs was reported to improve subscapularis TBJ repair.<sup>56</sup> The follow-up duration of the animal studies was usually less than 12 weeks and hence short (Supplementary material).

Five clinical trials reported the use of stem cells for the promotion of tendon repair (chronic tendinopathy, three; rotator cuff tear, two) with promising results (Supplementary material). There are two clinical studies reporting the safety of using allogeneic stem cells for the promotion of tendon repair. The injection of allogeneic ADSCs for the treatment of chronic lateral epicondylitis was reported to

be safe and effectively improved elbow pain, performance, and structural defects after 1 year in a small uncontrolled trial of 12 patients.<sup>57</sup> The ultrasound-guided injection of allogeneic human placenta-derived mesenchymal stromal cells was also reported to be safe in six patients with refractory Achilles tendinopathy at 4 weeks after administration.<sup>58</sup> Except the study by Hernigou et al,<sup>59</sup> the sample sizes in the other clinical studies were small and there were no control groups (Supplementary material).

Since ectopic bone was formed in some tendon samples after transplantation of BMSCs,<sup>42,43</sup> it is hypothesized that driving the MSCs toward the tenogenic lineage prior to transplantation may reduce the chance of ectopic bone and tumor formation as well as promote better tendon repair. Pretreatment of MSCs and MSCs derived from ESCs with growth factors (such as bone morphogenetic protein-12, connective tissue growth factor, platelet-rich plasma),<sup>60,61</sup> overexpression of key transcription factors (such as smad8, scleraxis [Scx], early growth response protein 1, mohawk [Mkx], membrane type 1-matrix metalloproteinase 1),<sup>62–68</sup> mechanical stimulation,<sup>69–71</sup> topographical cues of scaffolds (such as acellular tendon matrix, aligned chitosan-based ultrafine fibers),<sup>72,73</sup> or their combinations<sup>74</sup> have been used to promote tenogenic differentiation of stem cells prior to delivery. The current evidences suggested that most of these attempts were successful with better healing compared to the transplantation of untreated stem cells.

Pretreatment of BMSCs with bone morphogenetic protein-12 was reported to promote their tenogenic differentiation and efficacy of tendon repair after transplantation.<sup>60</sup> Platelet-rich plasma contains many growth factors and was reported to promote tenogenic differentiation of TDSCs.<sup>75</sup> Except one study,<sup>76</sup> combined treatment with stem cell and platelet-rich plasma was reported to enhance tendon repair compared to either one alone.<sup>61,77–80</sup>

Overexpression of key transcription factors of tendon development such as smad8, Scx, early growth response protein 1, and Mkx in MSCs was reported to promote their tenogenic differentiation and accelerate tendon and TBJ repair after transplantation (Supplementary material). In this regard, overexpression of a biologically active smad8 was reported to promote the tenogenic differentiation of a mouse MSC line that coexpressed the osteogenic gene (*BMP2*).<sup>62</sup> The transplantation of the resulting engineered cells to the injury site was reported to promote better healing in two rat Achilles tendon defect models.<sup>62,63</sup> The transplantation of MSCs overexpressing early growth response protein 1 was reported to promote junctional<sup>64</sup> and

mid-substance<sup>65</sup> tendon repair. The transplantation of MSC sheets overexpressing Mkx also promoted Achilles tendon repair in a mouse model.<sup>66</sup> While BMSCs overexpressing bone morphogenetic protein-13 did not improve supraspinatus TBJ healing,<sup>81</sup> BMSCs overexpressing Scx<sup>67</sup> or membrane type 1-matrix metalloproteinase 1 (a developmental protein)<sup>68</sup> were reported to improve healing in the same model.

Tendon is a mechanosensitive tissue. Appropriate tensile loading is required to maintain its composition and biomechanical properties. Tensile loading therefore has been used to promote the tenogenic differentiation and biomechanical properties of the tissue engineered constructs containing stem cells. Mechanical loading of BMSCs<sup>69,70</sup> and TDSCs<sup>71</sup> seeded in scaffolds prior to transplantation was reported to promote better tendon repair compared to the transplantation of the same unloaded cell constructs.

Biomaterial provides biological and architectural cues that can direct important cell behaviors and resulting tissue formation.<sup>82</sup> Different types and designs of scaffolds have been used as vehicles and/or as niche signal to drive tenogenic differentiation of stem cells with promising results in vitro and in animal studies. In this regard, acellular tendon matrix components, nanosized fibers, and aligned fibers have been shown in many studies to promote tenogenic differentiation of the seeded stem cells and the resulting constructs were reported to promote better tendon repair.<sup>72,73</sup>

Stem cells have also been used as carriers for growth factors to promote tendon repair (Supplementary material). The transplantation of BMSCs transfected with transforming growth factor- $\beta$ 1, but not vascular endothelial growth factor, promoted tendon repair.<sup>83</sup> Another study has reported that the transplantation of BMSCs had negative effects and overexpression of basic fibroblast growth factor in BMSCs had negligible effects on Achilles tendon healing.<sup>84</sup>

## Challenges and future research directions to enhance/optimize/standardize stem cell-based therapies to promote tendon repair

### Optimal stem cell source

Further studies are needed to compare if the transplantation of stem cells isolated from different tissues exhibited difference in outcomes of tendon healing in animal models. As ESC-based therapies are particularly susceptible to generating tumors if the graft contains any undifferentiated cells, strategy to effectively remove the undifferentiated cells is needed.

## Large animal models and clinical trials with long follow-up time

While the application of stem cells for the promotion of tendon healing is promising in small animal models, there have been few well-controlled large animal studies and clinical trials (Supplementary material). The follow-up duration in animal studies was usually short (usually 4–12 weeks). Further research on the efficacy and safety of stem cell-based therapy for tendon repair in well-designed large animal models with extended follow-up time and randomized controlled clinical trials is needed.

## Stem cell delivery methods

Studies comparing the use of different scaffolds for stem cell delivery in tendon repair are lacking. Further studies are needed to select the optimal scaffold for tendon repair. Delivery of stem cells via circulation is relevant for injury sites that are difficult to access such as the brain and the heart. Intralesional injection or transplantation is currently the commonly used administrative route for stem cell-based tendon repair as the tendons that are commonly injured (such as patellar tendon, Achilles tendon, and rotator cuff tendon) are located superficially. The administrative route may affect the distribution of available cells at the injury sites and clinical outcomes. The current limited evidence did not support the delivery of stem cells to the injured tendons via the circulatory system and regional perfusion.<sup>85,86</sup> Sole et al have reported that the MSC number in lesion site was lower when the cells were delivered via the circulatory system compared to the intralesional injection in an equine tendon injury model.<sup>85</sup> Moreover, arterial thrombosis was reported in the intravenously and intra-arterial perfused limbs.<sup>85</sup> Becerra et al have traced and compared the distribution of labeled BMSCs, that were delivered intralesionally, intravenously, or by regional perfusion, to the lesion site in an equine naturally-occurring tendon or ligament injury model.<sup>86</sup> Their results have shown that intralesional transplantation retained the highest number of BMSCs.<sup>86</sup> BMSCs were largely distributed to the lung fields and there were no detectable cells in the tendon lesions after intravenous injection.<sup>86</sup> This suggested that the cells could not “home” to the damaged tendon after intravenous administration. BMSCs could be detected in the lesion sites following regional perfusion though at a lower level compared to the intralesional injection. The cell labeling efficiency in the previous study was reported to be low and vary greatly (range from 1.3% to 18.5%, mean 7.2%).<sup>86</sup> The follow-up duration was also short (24–48 hours).<sup>85,86</sup> Further studies are needed to confirm the research findings

of the previous studies as well as to understand the safety and healing outcomes (eg, ectopic bone formation) of different delivery routes.

## Optimal cell density

There have been limited studies on the effects of cell density on tendon repair. One study has shown that collagen gel constructs seeded with higher density of BMSCs contracted faster and the seeded cells were better aligned after 3 days *in vitro*.<sup>87</sup> Awad et al have compared the healing effect of transplanting 1, 4, and 8 million cells/mL of autologous BMSCs seeded in collagen gel at the same cell to collagen ratio in a patellar tendon injury model.<sup>43</sup> They have reported that while the transplantation of BMSCs promoted tendon healing compared to natural repair, increasing the cell-seeding density did not produce additional histological and biomechanical benefits.<sup>43</sup> The authors have hypothesized that stress shielding produced by the tendon struts adjacent to the repair site might have reduced any benefits that would have accrued from increasing the seeding density of the implants *in vivo*.<sup>43</sup> The current limited data show that the initial seeding density is independent of ectopic bone formation as ectopic bone was formed in 28% of BMSC-treated tendons, regardless of cell concentration (1, 4, and 8 million cells/mL).<sup>43</sup> Nevertheless, the cell to collagen ratio and *in vitro* construct contraction may contribute to ectopic bone formation. Further studies in different tendon injury models are needed to identify the cell density that is most efficacious yet safe for tendon repair.

## Study on the fate and healing mechanisms of stem cells

The fate of stem cells after transplantation remains unclear. Some studies have reported the disappearance of the transplanted cells<sup>35,88</sup> while other studies have reported detecting the transplanted cells within the tendon injury site in the animal models but the follow-up time after transplantation was usually short.<sup>89,90</sup> MSCs might promote scarless tendon and TBJ repair via the secretion of a variety of bioactive factors that suppress inflammation, inhibit fibrosis and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of host reparative cells.<sup>35,37,40,91</sup> The transplantation of MSCs was reported to reduce the infiltration of inflammatory cells and promote tendon and TBJ repair in animal models.<sup>35,91</sup> The immunomodulatory role of MSCs on scarless tendon repair has been best demonstrated by the promotion of tendon and ligament repair with the injection of conditioned medium of amniotic membrane-derived mesenchymal

progenitor cells in horses<sup>40</sup> and the switching of macrophages from a pro-inflammatory to an anti-inflammatory phenotype after the addition of ADSCs in the macrophage and tendon fibroblast coculture.<sup>37</sup> Better understanding of the fate and healing mechanisms, including immunomodulatory roles, of stem cells after transplantation to the tendon injury site would facilitate treatment monitoring and enhancement.

Fetal tendon heals with scarless tissue formation. Many key transcription factors (such as *Sox* and *Mkx*) and growth factors (such as transforming growth factor- $\beta$ 2) for embryonic tendon development are also expressed in adult tendon.<sup>92</sup> Better understanding of the process of embryonic tendon development therefore can help the identification of novel therapeutic strategies, including stem cell-based therapy, to recapitulate critical aspects of tenogenesis and effectively direct the cells to differentiate and regenerate scarless tissues in adult tendon after injury.<sup>93,94</sup> Recently, the idea of mobilizing endogenous stem cells for regenerating tendon tissues has emerged.<sup>95,96</sup> Further understanding of the role of stem cells in natural tendon healing is warranted to explore strategies to promote tendon regeneration without cell transplantation.

## Manufacturing and regulatory considerations

The fact that the product of stem cell-based therapy is the living cells, not their isolated and enriched protein products, brings forward a different set of manufacturing and regulatory challenges. Specific issues associated with cell transplantation include scaling-up of production, biological or donor-to-donor variability, immunological responses to alloantigens, and tumorigenicity of the transplanted cells.

The number of stem cells required for tissue repair in the clinical setting is often greater than  $10^6$ – $10^7$  cells.<sup>57,58</sup> Safe, robust, and cost-effective strategies for scaling up the production of stem cells are required for translating the stem cell-based strategies from animal models to clinical trials. Optimization of cell culture system is needed for increasing the product quantity (ie, cell number) while maintaining its quality (eg, cell fate). Oxygen plays an important role in stem cell proliferation, maintenance, and differentiation.<sup>97,98</sup> Other parameters, such as pH, osmolarity, medium shear, and medium composition also have important effects on stem cell quantity and quality. These parameters need to be optimized and standardized to increase the stem cell yield while ensuring its safety.

Robust assays capable of product characterization, process validation and control, and predicting clinical safety

and efficacy are required for clinical application of stem cells. Identification of surrogate markers of cellular function and/or culture performance is required. This is not trivial. While the expression of transcription factors Oct4, *nanog*, and *sox2* are relatively reliable pluripotency markers (albeit intracellular) of ESCs and iPSCs, reliable markers of adult stem cells are rare. This is partly due to the poor understanding of the stemness functions of the current adult stem cell markers. Further studies on the stemness functions of markers commonly used for defining adult stem cells are needed. Although many MSC types were reported to exhibit low immunogenicity,<sup>33–35</sup> emerging studies also have reported that MSCs could adopt an immunogenic phenotype and stimulate immune cells.<sup>99</sup> Further research on the immunogenicity of MSCs is needed.

## Conclusion

Due to its relatively low cellularity and vascularity as well as the change in the tissue microenvironment after injury, tendons form scar tissue and ectopic bone after injury without regenerating the original tendon structure. Tissue engineering with stem cells offers the potential to replace the injured/damaged tissues with healthy and functional ones. The use of stem cells for tendon tissue engineering is advantageous compared to terminally differentiated cells as stem cells are pluripotent or multipotent, highly proliferative and synthetic, and can provide the appropriate signals to promote tendon regeneration compared to terminally differentiated cells. Moreover, stem cells can also be used as a vehicle for gene therapy and sustained delivery of bioactive factors for tendon repair. The previous animal studies have shown that stem cells and their modified counterparts were generally safe and effective for tendon repair, with the exception of the presence of ectopic bone and tumor in some studies. Many challenges have to be overcome before stem cells can be used as a safe and effective therapeutic option to promote tendon repair. Well-controlled large animal models with extended follow-up period and randomized controlled clinical trials are needed to evaluate the long-term safety and efficacy of stem cell-based products. The optimal scaffold, stem cell type, method of cell delivery, and cell density for tendon repair need further research. Translating stem cell-based therapies from bench to bed requires overcoming significant cell-manufacturing and regulatory challenges. Better understanding of the healing mechanisms of stem cell-based therapies and factors of embryonic tendon development would provide cues to promote tendon regeneration.

## Disclosure

The author reports no conflicts of interest in this work.

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