

# Tendon stem cells: experimental and clinical perspectives in tendon and tendon-bone junction repair

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## Summary

**Tendon and tendon-bone junction injuries, while heal, have high re-tear rates. Mesenchymal stem cells (MSCs) have great appeal for the promotion of tendon and tendon-bone junction healing because of their high proliferation rate, multi-potency and relative ease of isolation from various tissues. Tendon stem cells have been identified recently and could be an alternative new cell source for tendon and tendon-bone junction repair. In this review, we summarized the *in vitro* characteristics of tendon stem cells. The evidence supporting the potential use of these cells for tendon and tendon-bone junction repair was presented. In order to therapeutically apply tendon stem cells in the clinical settings, standardization of tendon stem cell culture is essential. Issues relating to the sources, purity, efficacy, safety and delivery of tendon stem cells for tendon and tendon-bone junction repair were summarized and discussed. The direction for future research was suggested.**

*Key words: tendon stem cells, tendon progenitors, tendon healing, tendon-bone junction healing, tissue engineering.*

## Epidemiology of tendon and ligament injuries

Tendon/ligament injuries are common in both the workplace and sport; with more than 30 million injuries occurring annually worldwide<sup>1</sup>. Achilles tendon and anterior

cruciate ligament (ACL) are the most common tendon and ligament sustaining acute injury, respectively. About 2 x 10<sup>5</sup> tendon and ligament repairs are performed annually in the United States<sup>2</sup>.

## Current treatment

Tendon and ligament injuries, whether chronic or acute, are commonly managed by conservative treatments or surgery. The effects of the commonly-used conservative treatments are frequently symptomatic, ineffective and the treatment time is long. If the conservative treatments fail, surgery, with the use of autografts, allografts, xenografts and prosthetic devices, is frequently required for repairing the injured tendon and ligament (replacement if it is an ACL tear)<sup>3,4</sup>. As many surgical tendon/ligament reconstructions, such as ACL reconstruction and rotator cuff tendon repair, require passing a tendon graft through a bone tunnel, it makes tendon and ligament repair more challenging as it requires the healing of two inhomogeneous tissues. Both tendon and tendon-bone junction tissues, while heal, have poor tissue quality and hence have high re-tear rates<sup>5-7</sup>. The treatment time is also long which prevents early return to sports.

## Tissue engineering for tendon and ligament regeneration

Tissue engineering is an interdisciplinary field that applies the principles of biology and engineering to the development of functional substitutes that restore, maintain or improve tissue function. It is a promising approach for the promotion of tendon and tendon-bone junction repair by improving the quality of healing for full restoration of function *per se* and reducing the chance of re-injuries. Cells, scaffolds and suitable biochemical and/or physio-chemical factors are combined for the functional restoration or regeneration of biological tissues in tissue engineering.

## Stem cell characteristics of tendon stem cells

Of the various cell types that are available for tendon and tendon-bone junction repair, mesenchymal stem cells (MSCs) are an attractive cell source as they have high proliferative potential and can differentiate into various cell types of the mesodermal lineage. MSCs were initially isolated in the bone marrow but later have also been isolated from many other tissues such as adipose tissue, umbilical cord, dental pulp, muscle and synovium. Recently, MSCs have also been identified from tendon tissues of

various species including human, mouse, rabbit, rat and horse, *in vitro*<sup>8-11</sup>. These tendon stem cells meet the MSC definition of the International Society for Cellular Therapy (ISCT)<sup>12</sup>. Previous studies showed that tendon stem cells were adherent to plastic, formed adherent colonies in cell culture and showed self-renewal potential<sup>8-10</sup>. They could differentiate into cells of osteogenic, chondrogenic and adipogenic lineages upon induction *in vitro* and could form tendon-like, bone-like, cartilage-like and tendon-bone junction-like tissues after subcutaneous transplantation in nude mouse or nude rat models<sup>8,9</sup>. Stem cells derived from the human patellar tendon proper expressed CD73, CD44, CD90 and CD105 but not CD34 and CD45 as shown by flow cytometry<sup>13</sup>. In another study, stem cells derived from rat patellar tendon showed null expression of CD11b and HLA-DR as well as very low level of CD79 (submitted unpublished results).

### Evidences supporting tendon stem cells as a good alternative cell source for tendon and tendon-bone junction repair

Although MSCs isolated from various tissues demonstrated some common stem cell characteristics, their stem cell properties were not identical<sup>14</sup>. As stem cells isolated from tendon tissue, the use of tendon stem cells for tendon and tendon-bone junction repair might be advantageous considering the fact that the tendon milieu is an ideal and familiar environment, which might promote the engraftment and differentiation of the transplanted cells. Moreover, we showed that tendon-derived stem cells (TDSCs) exhibited higher clonogenicity compared to BMSCs, indicating the ability to recruit more primitive stem cells in TDSC culture<sup>15</sup>. TDSCs also proliferated faster than BMSCs, which was beneficial for tissue engineering<sup>15</sup>. Similar finding was also reported by Bi et al.<sup>8</sup>. They found that human and mouse tendon stem/progenitor cells (TSPCs) proliferated faster than BMSCs isolated from the same person or animal<sup>8</sup>. The number of population doublings of mouse TSPCs was also higher than that of BMSCs, but this was not observed for human TSPCs<sup>8</sup>. Moreover, TDSCs expressed higher mRNA levels of tenogenic markers [scleraxis (*Scx*), tenomodulin (*Tnmd*) and extracellular matrix components of tendon [*Col1A1*, *Col1A1/C13A1* ratio, decorin (*Dcn*)] compared to BMSCs<sup>15</sup>. This finding was corroborated with the data of Bi et al.<sup>8</sup> which showed that mouse TSPCs expressed higher mRNA level of *Scx* than mouse BMSCs whereas human TSPCs expressed higher mRNA level of *TNMD* than human BMSCs. The results suggested that tendon stem cells might be a good cell source for tendon repair. Recently, we showed that the transplantation of TDSCs histologically and biomechanically promoted tendon repair<sup>16</sup> and the results were comparable to the use of BMSCs in a rat patellar tendon window injury model (unpublished results).

In addition to tendon repair, tendon stem cells might also be a good cell source for tendon-bone junction repair, which required the repair of bone and the interfacial fibrocartilage zone in addition to tendon. It has been reported that *Scx* regulated bone morphogenetic protein 4 (BMP4)

in tendon cells at their insertion site during deltoid tuberosity formation<sup>17</sup>, suggesting that tendon might also contribute to fibrocartilage formation at the tendon-bone junction. Rat TDSCs also exhibited higher chondrogenic and osteogenic markers at basal state, as well as chondrogenesis and osteogenesis upon induction, compared to BMSCs<sup>15</sup>. It is known that BMPs accelerated tendon-bone junction healing in animal models<sup>18-20</sup>. TDSCs expressed higher levels of BMP receptors and were more sensitive to BMP-2-induced osteogenic differentiation<sup>21</sup> compared to BMSCs. Higher osteogenic differentiation potential of mouse and human TSPCs compared to BMSCs was also observed in Bi et al.<sup>8</sup>'s study, but they did not compare the chondrogenic differentiation potential of TSPCs and BMSCs. Hence tendon stem cells might be suitable for tendon-bone junction repair with and without exogenous BMPs. It was likely that tendon stem cells were imprinted under the influence of local environmental niche so that they were more likely to produce tendon and junctional tissues. However, there has been no study to test the effect of tendon stem cells for tendon-bone junction repair. Further study is required to compare the effect of tendon stem cells and other MSCs for tendon-bone junction repair.

Below we discussed the practical issues pertaining to the therapeutic application of tendon stem cells for tendon and tendon-bone junction repair.

### Issues relating to the therapeutic application of tendon stem cells

#### Sources of tendon stem cells

It is difficult to get autologous tendon stem cells without causing donor site morbidity. Therefore only the use of allogeneic tendon stem cells with large quantity and with no immuno-rejection will justify their therapeutic use for tendon and tendon-bone junction repair. Low immunogenicity and immuno-modulatory effects of MSCs have been well-reported<sup>22-25</sup>. Recently, our research group showed that TDSCs lacked surface expression of MHCII, CD86 and CD80 (unpublished results).  $\gamma$ -interferon ( $\gamma$ -IFN) pretreatment did not increase MHCII and CD86 but slightly increased the expression of CD80. TDSCs did not induce proliferation and could escape the cytotoxic effect of lymphocytes isolated from the immunized animals. Incubation of TDSCs with the serum from the immunized animals also did not induce complement-dependent cell lysis and antibody recognition (unpublished results). There was no sign of inflammatory cells in the window wound after allogeneic TDSC transplantation<sup>16</sup>. Therefore, we believed that tendon stem cells were immune-privileged cells and might be used for allogeneic transplantation.

If an allogeneic source of tendon stem cells could be used for tissue engineering, then TDSCs could be easily isolated from the waste tendon tissue during tendon/ligament surgery, such as the residual tendon graft tissue in ACL reconstruction and the waste tendon tissue in total knee replacement. Our laboratory is currently collecting the discarded patellar and hamstring tendon tissues of ACL surgery for TDSC isolation<sup>13</sup>. A

clinical-grade Good Manufacturing Practice – compliant (cGMP) allogeneic tendon stem cellbank for clinical application could be established. The use of allogeneic tendon stem cells for tendon and tendon-bone junction repair avoids delayed treatment to patients compared with the use of autologous cell source. Unlike bone marrow aspiration for the isolation of BMSCs, the harvest of residual tendon tissue does not require a separate surgery and does not impose additional pain to donors. The concentration of BMSCs obtained per milliliter decreased with increased volume of aspirated marrow for each puncture because of dilution of bone marrow sample with peripheral blood<sup>26</sup>. This would not be an issue for tendon stem cell isolation.

#### *Purity and safety of tendon stem cell culture*

Tendon stem cells, like other MSCs, are mainly isolated by an enrichment procedure. They are hence a heterogeneous cell population with variation in stem cell characteristics and might also be contaminated with undesirable cell types, such as tenocytes. The resulting variability limits standardization of tendon stem cell-based repair strategies and also impedes the comparison of treatment outcomes. The development of quality controls for the isolation of pure, safe and effective tendon stem cell populations is hence vital for their routine use in the clinical practice.

Although many different MSC markers have been reported, unique markers for the unambiguous identification of MSCs *in vitro* and *in vivo* are yet to be discovered. Commonly-used stem cell surface markers such as CD44, CD90, CD73, CD29 and CD105 were not specific to MSCs and were also expressed by fibroblasts<sup>27,28</sup>. Zhang and Wang<sup>9</sup> characterized tendon stem cells and tenocytes *in vitro* and reported that tendon stem cells (TSCs), but not tenocytes, possessed multi-lineage differentiation potential. TSCs expressed stem cell marker proteins Oct-4, SSEA-4 and nucleostemin whereas tenocytes expressed none of these markers<sup>9</sup>. They therefore suggested using these markers to differentiate TSCs *in situ*. The discovery of surface markers that could specifically differentiate tendon stem cells from tenocytes and possibly other sources of MSCs would ensure the consistent isolation of a pure and effective tendon stem cell population for clinical applications. The genomic stability of tendon stem cells is essential for their safe application in tissue engineering. We (unpublished results) and others<sup>8,9</sup> did not observe tumor formation after subcutaneous transplantation of tendon stem cells in nude animals. Still, the use of karyotyping to demonstrate the stability of the genome of tendon stem cells during *in vitro* culture is essential, prior to their clinical use.

#### *In vitro expansion of tendon stem cells*

Tendon stem cells only made up 1-4% of the total nucleated cells in tendon<sup>8,10</sup> and the number of TSPCs isolated from tendons was markedly reduced with aging<sup>29</sup>. The proliferation rate of aged TSPCs was also reported to be lower compared to young TSPCs<sup>29</sup>. Aged TSPCs expressed lower basal and TGF- $\beta$ 3-induced mRNA expression of *Scx* and *Tnmd*, but higher adipogenic differ-

entiation potential following induction, compared to young cells<sup>29</sup>. Hence, *in vitro* culture and expansion of tendon stem cells are required for obtaining enough cells for tendon and tendon-bone junction repair. However, only the early passages of tendon stem cells might be useful for tendon and tendon-bone junction repair as long-term *in vitro* culture of rat TDSCs resulted in cellular senescence and loss of multi-lineage differentiation potential<sup>30</sup>. Therefore, strategies that can promote the expansion while maintain the stemness properties of tendon stem cells are essential. We showed that culturing TDSCs under a hypoxic environment (2%O<sub>2</sub>) enhanced clonogenicity and proliferation as well as reversibly suppressed the multi-lineage differentiation potential of TDSCs upon induction<sup>13</sup>. Culturing of TSCs on/in decellularized tendon matrix increased their proliferation, self-renewal capacity and multipotency compared to culturing TSCs on the plastic culture surface<sup>31</sup>. Implantation of TSCs in decellularized tendon matrix promoted tendon-like tissue formation while implantation of TSCs alone did not result in the formation of tendon-like tissue<sup>31</sup>. However, the fate of transplanted TSCs was not reported in this study, hence it was not clear if the transplanted cells without scaffold were still in place after surgery.

#### *Tenogenic differentiation of tendon stem cells*

Although BMSCs have been reported to promote tendon and tendon-bone junction healing in various animal models<sup>32-34</sup>, there were also reports of negative results<sup>34</sup>, risk of ectopic bone formation<sup>35</sup> and tumor induction<sup>36</sup> following transplantation of BMSCs. Therefore, strategies that promote the *in vitro* tenogenic differentiation of BMSCs prior to cell transplantation into tendon or tendon-bone injury sites were advocated<sup>37-41</sup>. Improvement in tendon and tendon-bone junction healing was reported after genetic modification or pre-treatment of MSCs with these factors<sup>42-44</sup> except in one study<sup>45</sup>. Similar to BMSCs, tendon stem cells theoretically could also induce formation of unwanted tissue after transplantation given their high multi-lineage differentiation potential<sup>8,15</sup>. We did not observe an increase in the risk of ectopic bone formation after transplantation of allogeneic TDSCs in a patellar tendon window injury rat model which showed ossification at the late stage of tendon healing in 3/6 of samples in histology at week 12<sup>46</sup>. Whether the use of tendon stem cells driven to the tenogenic lineage would further promote tendon healing and reduce the risk of ectopic bone formation in the patellar tendon window injury rat model requires further research. Various growth factors such as GDF-5, GDF-6 and GDF-7 as well as *Scx* over-expression have been reported to promote the tenogenic differentiation of BMSCs or adipose tissue-derived MSCs (AdMSCs)<sup>37-41</sup>. Whether these factors would have similar tenogenic effects on tendon stem cells were not clear. Zhang and Wang<sup>47</sup> reported that platelet-rich plasma-clot releasate (PRCR) could promote the differentiation of TSCs into active tenocytes with high proliferation rate and collagen production capability.

#### *Tendon stem cell delivery*

MSCs have homing ability to some tissues, particularly when the tissue is injured or under pathological condi-

tions<sup>48</sup>. After tendon and tendon-bone junction injuries, MSCs could be delivered to the target site for repair either by systematic infusion or local delivery. Dudhia et al.<sup>49</sup> compared the routes of administration of BMSCs for the repair of equine tendinopathies or desmopthies. They reported that intra-lesional injection of BMSCs retained the higher number of cells at the injury site, followed by regional perfusion. Intravenous injection of BMSCs resulted in the distribution of cells to the lungs, without detectable level of BMSCs in the tendon lesions. The best temporal therapeutic window for systematic administration of MSCs has to be identified as the recruitment and treatment effect of MSCs was reported to be time-dependent; and was effective only when MSCs were injected at the time when stromal cell-derived factor-1 (SDF-1) was expressed in the injured tissue at the early phase post-myocardial infarction<sup>50</sup>. There has been no study on the homing ability of systematically-injected tendon stem cell safter tendon and tendon-bone junction injuries. However, if surgery is required after injury, implantation of tendon stem cells in a bio-scaffold seems to be a straight-forward method. Zhang et al.<sup>31</sup> reported that acellular tendon matrix was a good biomaterial for the culture and delivery of tendon stem cells as it preferentially promoted tenogenic differentiation of TSCs *in vitro* and hence, tendon-like tissue formation, *in vivo*. Further research is needed to identify a good biomaterial for the delivery of tendon stem cells to the tendon or tendon-bone interface. Figure 1 shows e schematic diagram summarizing the studies required for traslating the appli-

cation of tendon stem cell for tendon and tendo-bone junction repair.

### Conclusion and direction of future research

In conclusion, we have summarized the *in vitro* characteristics of tendon stem cells. Given their favorable *in vitro* stem cell characteristics, tendon stem cells might be a good cell source for tendon and tendon-bone junction repair. The effect of tendon stem cells in tendon repair has been reported but there has been no study on their effects in tendon-bone junction repair. As tendon stem cells were not immunogenic, an allogeneic cell source could be used. We discussed the practical issues when applying tendon stem cells for tendon and tendon-bone junction repair, including the specific identification and genomic stability of tendon stem cells, both have not been solved and required further research. This information is essential for the future clinical application of tendon stem cells in tendon and tendon-bone junction repair. Some strategies for the *in vitro* expansion of tendon stem cells have been reported but new and better strategies to promote the expansion of tendon stem cells *in vitro* are needed. Whether tenogenic differentiated tendon stem cells would promote better tendon and tendon-bone junction healing in terms of rate and quality needed further research. The study on the biomaterials for the delivery of tendon stem cells is still in the infancy. Further research is needed to identify a good biomaterial for the

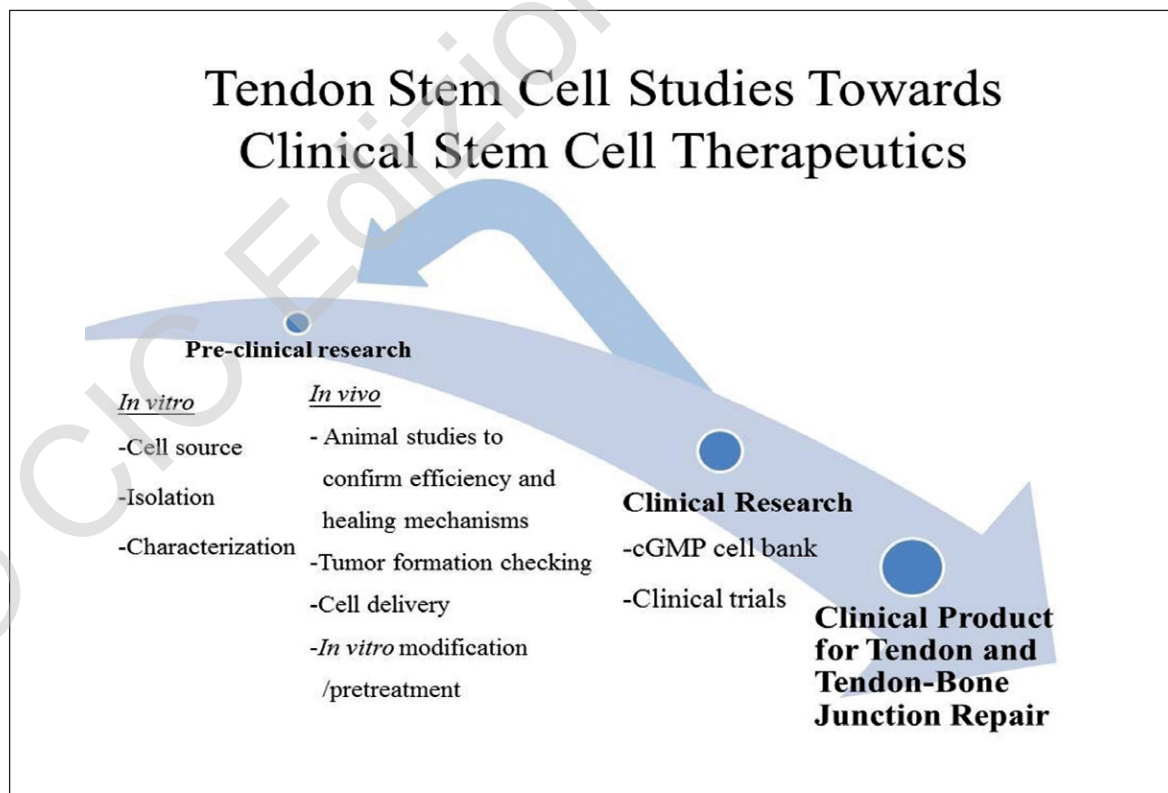


Figure 1. Schematic diagram showing the studies required for translating the application of tendon stem cells for tendon and tendon-bone junction repair.



delivery of tendon stem cells to the tendon or tendon-bone interface.

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### Conflict of Interest

We don't have any conflict of interest.

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