

# Musculoskeletal diseases—tendon

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**Introduction:** Tendons establish specific connections between muscles and the skeleton by transferring contraction forces from skeletal muscle to bone thereby allowing body movement. Tendon physiology and pathology are heavily dependent on mechanical stimuli. Tendon injuries clinically represent a serious and still unresolved problem since damaged tendon tissues heal very slowly and no surgical treatment can restore a damaged tendon to its normal structural integrity and mechanical strength. Understanding how mechanical stimuli regulate tendon tissue homeostasis and regeneration will improve the treatment of adult tendon injuries that still pose a great challenge in today's medicine.

**Source of data:** This review summarizes the current status of tendon treatment and discusses new directions from the point of view of cell-based therapy and regenerative medicine approach. We searched the available literature using PubMed for relevant original articles and reviews.

**Growing points:** Identification of tendon cell markers has enabled us to study precisely tendon healing and homeostasis. Clinically, tissue engineering for tendon injuries is an emerging technology comprising elements from the fields of cellular source, scaffold materials, growth factors/cytokines and gene delivering systems.

**Areas timely for developing research:** The clinical settings to establish appropriate microenvironment for injured tendons with the combination of these novel cellular- and molecular-based scaffolds will be critical for the treatment.

**Keywords:** tendon injury/tissue engineering/regenerative medicine/stem cells/scleraxis/mechanical force

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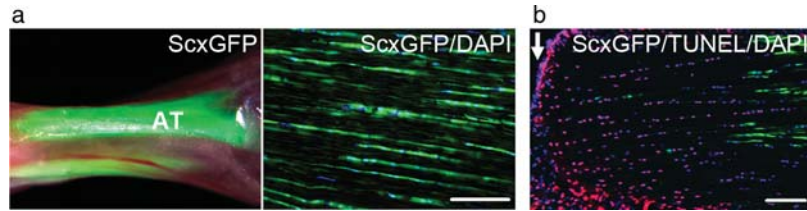
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## Tendon physiology

Tendon, a fibrous connective tissue made of specialized fibroblasts called 'tenocytes' and an abundant collagenous extracellular matrix (ECM), is a tissue whose physiology and pathology is heavily dependent on mechanical stimuli.<sup>1</sup> Tendons establish specific connections between muscles and the skeleton by transferring contraction forces from skeletal muscle to bone, thereby allowing body movement.<sup>2</sup> Tendons exhibit high mechanical strength, good flexibility and an optimal level of elasticity to perform their unique role. The tensile strength of a tendon is related to its thickness and collagen content: for example, a tendon with an area of 1 cm<sup>2</sup> is capable of bearing 500–1000 kg.<sup>3</sup> Tendons have relatively few blood vessels and function at a low metabolic rate. Tendons receive oxygen and nutrients from three main sources: internally via the myotendinous junction and osteotendinous junctions, and externally through the paratenon or the synovial sheath.<sup>4</sup>

## Tendon development and adult homeostasis

During embryonic development, tenocytes originate from mesodermal compartments, as do skeletal myoblasts, chondrocytes and osteoblasts.<sup>5</sup> Some of the multipotent mesenchymal progenitor cells that arise from these compartments express the basic helix-loop-helix transcription factor scleraxis. However, once they are committed to become cells making up a specific tissue, only tendon progenitor cells and tenocytes retain the ability to express scleraxis. Therefore, scleraxis is a highly specific marker of tenogenic cells and mature differentiated tenocytes.<sup>6,7</sup> The *scleraxis* gene is thus the first master gene found to be essential for establishing the tendon lineage during development.<sup>8</sup> Tenomodulin is a type II transmembrane glycoprotein. Its robust expression is induced in mouse tendons in a late (embryonic day [E] 17.5) developmental phase and is also observed in adult tendons showing that tenomodulin is a marker of mature (differentiated) tenocytes.<sup>9</sup> *In vitro* experimental evidence shows that the genes composed of tendon-specific ECM are tightly regulated in tenocytes by mechanical forces.<sup>2</sup> Very recently, tendon stem/progenitor cells have been discovered in human and mouse tendon, and the proteoglycans biglycan and fibromodulin have been identified as essential elements in a micro-environment to keep phenotypes and differentiation of stem/progenitor cells.<sup>10</sup> However, the roles of these stem/progenitor cells in adult tendon homeostasis and/or wound healing remains unknown.



**Fig. 1** Tensile loading plays a crucial role in tenocytes. (a) Achilles tendons in adult *Scleraxis-GFP* transgenic mice. Left panel: under fluorescence stereomicroscope; right panel: under microscope with GFP/ultraviolet (UV) filters to show scleraxis-GFP (green) and nucleus [4'6-diamidino-2-phenylindole (DAPI) blue]. AT, Achilles tendon. Bar = 100  $\mu$ m. (b) Analysis of cell death at 2 h after complete transection of adult Achilles tendon in *Scleraxis-GFP* transgenic mice. Arrows indicate the transection edge of tendons. Note that the expression of scleraxis-GFP (green) is diminished and cells positive by terminal deoxynucleotidyl transferase dUTP nick-end labeling assay (TUNEL assay: a marker for cell death, red) are evident in the transected Achilles tendon. Bar = 100  $\mu$ m.

## Mechanical force and tenocytes

Since tendon tissues are constantly exposed to variable transmittal forces from skeletal muscles, our laboratory examined the functional links between mechanical forces and tenocyte phenotypes using scleraxis as a tenocyte marker. We utilized a transgenic mouse strain, which expresses the green fluorescent protein (GFP) marker driven by the *scleraxis* gene in a way that GFP is produced in a pattern that mimics its expression in the body<sup>6</sup> (*Scleraxis-GFP* transgenic mice were kindly provided by Dr Ronen Schweitzer, Research Division, Shriners Hospital for Children, Portland, OR, USA). The vast majority of tenocytes show robust expression of scleraxis-GFP in adult tendon tissues under the fluorescence microscope (Fig. 1a). Strikingly, the sudden interruption of continuous tensile loading, such as by complete transection tendon injury, leads to a decreased expression of scleraxis and tenocyte death (Fig. 1b). Thus, these findings indicate a critical role for mechanical forces in adult tendon homeostasis and strongly suggest new directions for therapy following tendon injuries.<sup>11</sup>

## Tendon injury

### *Incidence of tendon injury*

Soft-tissue injuries, including injury to tendon, ligament or meniscus, can induce abnormal joint motions and altered loading in the short term and they could contribute to degenerative joint disease and osteoarthritis in the long term.<sup>12</sup> These injuries can be acute or chronic and are caused by intrinsic or extrinsic factors, either alone or in

combination.<sup>4</sup> Acute tendon injury interrupts tendon continuity with consequent disruption of ECM architecture and dramatic loss of transmittal forces from skeletal muscle.<sup>4</sup> Tendon injuries represent a serious and still unresolved problem. More than 130 000 patients per year undergo tendon-related surgery in the USA.<sup>13</sup> The tendons most frequently affected are shoulder rotator cuff (51 000 cases), Achilles tendon (44 000 cases) and patellar tendon (42 000 cases).<sup>13</sup> Injuries to Achilles tendon, patellar tendon, hand flexor tendon and shoulder rotator cuff have clinical importance since they can lead to loss of muscle function, significant disability, joint instability and secondary osteoarthritis, adversely affecting a patient's activities of daily living and quality of life. The incidence of tendon injury has increased in recent years as the number of aging adults continues to grow.<sup>14</sup> The altered activity of mechanical loading, and vasculature and angiogenesis are suggested to play a significant role in degenerative tendon diseases.<sup>15,16</sup>

### *Tendon healing*

Tendon wound healing involves regeneration of tenocytes and reconstruction of dense collagen fibrils, and the tendon repair process in transected experimental animal tendons is known to involve three overlapping phases, as for other organs/tissues.<sup>4,13</sup> An initial, inflammatory phase occurs until Day 2 after injury. It involves extensive cell death in the injured area and subsequent inflammatory cell infiltration. A second, proliferative phase starts at Day 3. It involves cell migration into the injured area, extensive proliferation and production of collagen fibrils. A third, remodeling phase occurs from 6 weeks on. This phase can be divided into a consolidation stage, from 6 to 10 weeks after injury, and a maturation stage, after 10 weeks. It is characterized by decreased cellularity and collagen synthesis, and the alignment of tenocytes and collagen fibrils in the direction of stress. ECM-remodeling during tendon wound healing follows in general the same processes as in other tissues, i.e. in an early stage, provisional matrix formation by the plasma proteins fibrinogen and fibronectin, followed by replacement of the provisional matrix by collagen fibrils.<sup>2,4</sup>

In the inflammatory phase, vasoactive and chemotactic factors such as cytokines and growth factors are released and lead to an increased vascular permeability, initiation of angiogenesis and stimulation of tenocyte proliferation. In particular, various growth factors/cytokines play a number of important roles, including stimulation of tenocyte proliferation, cell migration to the wound and synthesis of the new ECM during tendon healing.<sup>17,18</sup> In the proliferation phase, two

mechanisms, intrinsic and extrinsic mechanisms, are likely to contribute to the healing process. The intrinsic mechanism involves the proliferation of tenocytes from the tendon and epitenon. These tenocytes contribute to synthesize the new ECM, which consists largely of collagens and glycosaminoglycans. Such intrinsic healing results in improved biomechanics and fewer post-injury complications. In contrast, the extrinsic mechanism involves the migration of inflammatory cells and fibroblasts from the overlying sheath and synovium into the wounded site.<sup>4,19</sup> This often causes scar tissue and results in adhesion formation, which disrupts tendon gliding. Although tendon healing follows the same process as that in various connective tissues, including skin and muscle, tendon tissues heal more slowly than other connective tissues, probably because of the low metabolic rate of tendons and their dense and hypocellular composition (only 5% of the volume is occupied by cells).<sup>4,5</sup> Furthermore, ECM remodeling such as increasing diameters and cross-linking of collagen fibrils occurs over a period of up to 2 years following tendon injury.<sup>3</sup> Despite such remodeling, the biochemical and mechanical properties of healed tendons never match those of intact ones.<sup>3</sup> The final tensile strength in the healed tendons is eventually reduced to ~30% the strength of intact tendons.<sup>20</sup>

## Therapies in tendon in injury

### *General concepts in therapies in tendon injury*

Clinically, two main strategies for treating injured tendons are followed:<sup>21</sup> (i) leave them untreated to heal naturally or (ii) perform surgery for primary repair of injured tendons using various techniques (Table 1). These treatments are determined in general according to clinical factors, such as age, tendon location, mode of injury and size of defect. Surgical treatment of an acute Achilles tendon rupture has been considered superior to non-operative treatment to prevent the risk of re-rupture.<sup>22,23</sup> However, investigators in a recent multicenter randomized trial (NCT00284648 in ClinicalTrials.gov) reported that similar clinical outcomes are observed between (i) non-operative groups with accelerated functional rehabilitation and (ii) operative groups.<sup>24</sup> Thus, the treatment for acute Achilles tendon rupture remains inconclusive.<sup>22,24</sup> Furthermore, for injuries to the hand flexor tendon or shoulder rotator cuff, long-term clinical outcomes of primary repair remain unsatisfactory.<sup>13,25</sup> Despite improved surgical techniques and advances in postoperative therapy regimens, these treatments still have potential complications such as re-rupture of the repair site or the formation of restrictive adhesions.<sup>25,26</sup> Recently, the

**Table 1** Characteristics for treatment in tendon injury.

Treatment type	Indication	Advantages	Disadvantages
Immobilization	Acute Achilles tendon rupture	No surgical complication	Failure to heal
Plaster Brace	Tendinopathy		Rerupture Muscle atrophy Tendon atrophy Limitation of range of motion Decreasing of tensile strength Suture failure
Primary repair	Most acute tendon injury	First clinical choice	
	Chronic tear of rotator cuff	Recovery of muscle contraction	Rerupture  Inability to achieve functional results Postoperative adhesion Infection
Tendon transfer	Large tendon defect Primary repair failure	Filling of tissue defect Recovery of muscle contraction	Suture failure Rerupture
	Chronic tendon injury		Inability to achieve functional results Postoperative adhesion Infection Altered biomechanics of joint
Tendon graft	Large tendon defect	Filling of tissue defect	Suture failure
Autograft	Primary repair failure	Recovery of muscle contraction	Rerupture
Allograft	Chronic tendon injury		Inability to achieve functional results
Xenograft	Destructive disease		Postoperative adhesion
Synthetic material	Congenital disorder		Infection  Limited availability of material Altered biomechanics of joint Deficiency of biocompatibility Immunologic rejection Antigenic reaction Disease transmission

biological grafts, such as autologous fascia, porcine small intestinal submucosa or synthetic materials, have been developed and used in tendon graft procedures to fill large tissue defects (Table 2). However, no graft method exists to restore a damaged tendon to its normal function. Grafting poses several potential complications, including increased inflammatory responses, antigenic reactions, failure at the fixation sites and deficiencies in long-term biocompatibility.<sup>25,26</sup> Thus, current knowledge of the biology in tendon healing has yet to lead to clinically successful strategies for treatment.<sup>19</sup> New treatment modalities are required to promote the regeneration of tendon tissues.

**Table 2** Scaffold materials for tendon injury.

Biologic (naturally occurring)	
Human tissue	Dermis Dura mater
Animal tissue	Porcine small intestinal submucosa Porcine dermis Type I collagen
Biopolymers	
Synthetic (manufactured)	
Resorbable	Polyethylene Polyglycolic acid Polylactic acid Poly- <i>N</i> -acetyl- <i>D</i> -glucosamine
Non-resorbable	Carbon fibers Dacron® Nylon Polyacrylamide Silicone Teflon® Silk

### *New treatment strategies for tendon healing*

To design efficient strategies to enhance tendon repair following injury, it is essential that scientists and clinicians understand the cellular and molecular mechanisms responsible for the development, homeostasis, regeneration and repair of tendons. To date, however, the molecular mechanisms underlying tendon repair remain largely unknown. The target molecules for tendon repair are based on knowledge from general wound healing, not on results generated specifically from analysis of tendon healing.<sup>27</sup>

Tissue engineering is an emerging technology that offers a novel approach for treating tendon injuries and restoring tissue and joint function.<sup>28</sup> The most common tissue-engineering principles are (i) the use of healthy multipotent cells that are non-immunogenic, easy to isolate and highly responsive to distinct environmental cues; (ii) the development of carrier scaffolds that provide short-term mechanical stability of the transplant as well as a template for spatial growth of the regenerating tissue; and (iii) the delivery of growth factors that drive the process of cell differentiation and maturation.<sup>25</sup>

Orthopedic tissue engineering comprises elements from the fields of cell biology, materials science, mechanical engineering and surgery.<sup>12</sup> Various types of scaffolds, such as naturally occurring ECMs as well as cell-based strategies have been developed<sup>12,25,26,29–33</sup> (Table 2). Mesenchymal stem cells (MSCs) are becoming a subject of increasing interest because of their potential utility in tissue-engineering applications<sup>34,35</sup> (Table 3). MSCs exist in adult bone marrow and can be induced to form different mesenchymal tissue lineage cells such as

**Table 3** New modalities for treatment in tendon injury.

Treatment type	Methods of delivery	Advantages	Disadvantages
<b>Scaffolds</b>			
Biologic material	Direct implantation	Abundant supply (type I collagen)	Limited clinical applications (autograft) Limited recovery of strength
		Relatively low complications (type I collagen)	Potential complications as in Table 1
Synthetic material	Direct implantation	Abundant supply	Limited recovery of strength Limited regeneration Potential complications as in Table 1
Cell therapy			
Tenocyte	Direct implantation with collagen materials	Differentiated cell	Limited cellular source  Low proliferative ability Low morbidity
Mesenchymal stem cell	Direct implantation with collagen materials	Excellent regenerative capacity	Unclearness of mechanisms in differentiation
		High proliferative ability	Low morbidity Invasive procurement procedure Low yielding
Growth factors	Direct administration	Easy administration	Unclearness of growth factor stability Unclearness of effective concentration
Gene therapy			
Viral method	Direct viral infection	High transduction efficiency	Inflammatory response
Non-viral method	Direct administration with liposomes	Transient expression	Non-specific infection
		Low pathogenic response	Relatively low transduction efficiency

chondrocytes, adipocytes or osteoblasts. In fact, MSCs-based scaffolds have been tried in animal models of tendon wound healing.

### Cellular scaffold-based therapy

#### *Scaffolds*

The underlying concept for tissue engineering technologies has been changing. Traditionally, a graft was composed of some material (such as nylon or silk) meant solely to fill the tissue defect. Nowadays, implants are expected to serve as 'biocompatible scaffolds' (natural or synthetic materials that can be replaced by host tissues without undesirable responses). These biocompatible scaffolds are suitable as vehicles for implanted cells, the delivery of growth factors, or the transfer of



genes<sup>25,26</sup>. Both biologic and synthetic materials are used to create scaffolds for tendon reconstruction with a three-dimensional biocompatible construct that serves as a temporary or permanent implant<sup>26</sup>. As described, injured tendons have very limited spontaneous healing capabilities. Thus, ideal scaffold materials need to play at least two essential roles: to stimulate regeneration (including proliferation and differentiation of cells) at implanted sites and to establish the specific composition and structure of an ECM that can then provide an appropriate microenvironment for regenerating cells. The major ECM component in tendons is type I collagen. The advantages of using type I collagen for tendon reconstruction include its strength, capacity to resorb and ability to induce the alignment of host connective tissues.<sup>26</sup> Scaffolds of type I collagen cross-linked with glutaraldehyde or carbodiimide are used in research to regenerate tendon tissue because of the low antigenicity and strength.<sup>26,36</sup> Indeed, they have improved graft strength in a rabbit Achilles tendon model.<sup>36</sup> Synthetic non-resorbable materials, including nylon, silk and carbon, are not biocompatible because of host foreign body responses and late mechanical failure.<sup>26</sup> To circumvent these problems, synthetic resorbable materials have been developed using polyglycolic acid or polylactic acid.<sup>25</sup> They can be fabricated into three-dimensional scaffolds of variable structure and porosity with a correspondingly wide range of mechanical and degradation properties.<sup>25</sup> Unfortunately, some synthetic resorbable scaffolds alter the mechanical properties of the repaired tendon, lose strength and integrity over time, limit tendon ingrowth, cause abrasions of surrounding tissues, enhance the inflammatory response and cause undesirable scar formation around the repair site.<sup>26</sup> A study in a goat shoulder rotator-cuff repair model indicates that the polylactic acid-scaffold does not show significant increase in the load-to-failure strength, even though the polylactic acid patch is occupied by cellular fibrous tissues.<sup>37</sup> Therefore, despite their potential roles in tendon reconstruction, further investigation will be necessary to find an alternative to natural materials.

#### *Cell-based therapy*

Cell-based therapy is also a novel technique to improve the composition, structure and biomechanical properties of new tendon tissue: cells are initially seeded onto scaffolds, and then they are delivered to the injured sites as cell- and scaffold-combined materials.<sup>26</sup> To date, several different combinations of cell types and biomaterial scaffolds have been used in experimental animal models (including MSCs-type I collagen gel, MSCs-knitted polylactide-co-glycolide matrix, tenocytes-non-woven polyglycolic acid fibers), and they have the capacity to enhance tendon formations.<sup>30-33,38</sup> In these biomaterial

scaffolds, a plenty of materials such as collagen gel or synthetic biodegradable polymers are commercially available. On the other hand, cells seeded on such a scaffold need to proliferate rapidly *in vitro* to provide adequate numbers for *in vivo* implantation.<sup>25</sup> An important prerequisite for cell-based therapy is the successful isolation and selection of appropriate cells.<sup>25</sup> A tenocyte-based method is one of the potential cell-based therapies, but a number of concerns still limit the practicality of its use: (i) a limited availability of donor sites tenocytes from which tenocytes may be obtained for implantation, (ii) the time requirements for lengthy *in vitro* culture to expand the number of cells and (iii) the morbidity of tenocytes themselves.<sup>39</sup> To circumvent the negative impact of this tenocyte-based method, MSCs have been investigated as an alternative source for tendon engineering. MSCs, which show an excellent capability for regeneration and rapid proliferation, have the potential to differentiate into a spectrum of specialized mesenchymal tissues, tendon, ligament, bone, cartilage, muscle, fat and marrow stroma.<sup>25</sup> In addition, MSCs can be relatively easily isolated from bone marrow, but they are also found in muscle, adipose tissue, skin and around blood vessels.<sup>40</sup> The ability of MSCs for tendinogenic differentiation has been documented in several studies.<sup>31–33</sup> In fact, recruitment of MSCs to accelerate repair and tissue regeneration was shown *in vivo* in a rabbit tendon tissue model.<sup>32</sup> However, no significant differences were observed in mechanical properties between MSC-transplanted and non-transplanted repaired tissues. Moreover, 28% of MSC-treated tendons developed foci of ectopic bone, whereas no bone formed in naturally healing contralateral controls.<sup>29,41</sup> These studies clearly indicate that the determination of an appropriate MSC microenvironment for tenocyte differentiation is a critical issue that needs further investigation. We also need to take into consideration several more issues relating to the clinical application of MSC-based therapy: long-term safety of the patient, large-scale culture and storage of cells, ideal scaffold materials, optimal cell seeding conditions and an alternative mode of applying MSC-material composite to the injured site.<sup>4,25</sup>

### **Molecular-based therapy**

#### *Growth factors and cytokines*

Growth factors/cytokines represent one of the largest molecular families involved in the wound healing process, and a considerable number of studies have been undertaken in an effort to elucidate their many functions and behaviours during healing progression.<sup>17</sup> Several molecules have been identified as key factors during the repair process of tendons, including transforming growth factor- $\beta$  (TGF- $\beta$ ), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF),

vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and growth and differentiation factor (GDF)-5 through 7.<sup>26</sup> Since TGF- $\beta$  regulates a wide variety of cellular processes, including the expression of scleraxis during tendon formation in embryonic development,<sup>42</sup> such multifunctional aspects of TGF- $\beta$  have been extensively studied in relation to adult tendon injury and homeostasis. The expression levels of TGF- $\beta$  in adult tendons are dramatically upregulated in a short time after injury, and TGF- $\beta$  initiates an inflammatory response to tissue damage.<sup>17</sup> In contrast, TGF- $\beta$  upregulates the production of ECMs, which results in excessive scar formation. Indeed, the local administration of a neutralizing antibody of TGF- $\beta$  can diminish excessive production of ECM and improve the postoperative range of motion in a rabbit model of complete transection of the hand flexor tendon.<sup>43</sup> Thus, such contradictory functional aspects of TGF- $\beta$  make it difficult to rely on TGF- $\beta$  for clinical use in tendon healing.<sup>3</sup> IGF-1 stimulates synthesis of DNA, collagen and proteoglycans, as well as tenocyte proliferation and migration *in vitro*.<sup>44</sup> IGF-1 also acts synergistically with PDGF to stimulate tenocyte migration.<sup>44</sup> A study in a rat Achilles tendon transection model indicates that the injection of IGF-1 at injured sites accelerates functional recovery of Achilles tendon.<sup>45</sup> GDF-5, -6 and -7 (members of the TGF- $\beta$  superfamily that are related to bone morphogenetic proteins) can induce neotendon formation, as assessed by histochemical analysis when injected at subcutaneous sites in rats.<sup>18</sup> Another study shows that the injection of GDF-5, -6 or -7 into injured Achilles tendons in rats results in a significant dose-related increase of mechanical properties in rat Achilles tendon.<sup>46</sup>

Some success has been achieved utilizing single growth factors as therapeutics.<sup>17</sup> Direct injection of a growth factor at the injured site may give a temporary boost of a single healing signal but has only limited effect on the final outcome.<sup>17</sup> The combination of patients' own growth factors to promote healing in injured tissues is a potentially very fruitful area of research.<sup>17</sup> Platelet-rich plasma (PRP), easily harvested from whole blood by a few centrifugation steps, contains autologous growth factors such as PDGF, TGF- $\beta$ , IGF-1 and -2 and bFGF.<sup>47</sup> Postoperative direct injection of PRP significantly improves mechanical strength and stiffness in a rat Achilles tendon repair model.<sup>48</sup> Recently, there has been increasing interest in the field of sports medicine to facilitate healing and earlier return to activity after tendon and ligament injury.<sup>49</sup> Several clinical trials investigating the efficacy of PRP treatment have been performed for Achilles tendon rupture (NCT00731068 in ClinicalTrials.gov) and rotator cuff injury (NCT01000935; NCT01152658; NCT01170312 in ClinicalTrials.gov). However, recent randomized

clinical trials indicate that PRP treatment has no significant effect on the healing of tendon and ligament injuries.<sup>49,50</sup>

### *Gene therapy*

A variety of gene transfer methods have been used both *in vitro* and *in vivo* to induce local production of growth factors such as GDF-7 or PDGF by means of viral (e.g. adenovirus, retrovirus) or non-viral vectors (e.g. liposomes) to accelerate the tendon repair process.<sup>51,52</sup> The use of a non-viral vector-mediated delivery is less pathogenic because of the absence of viral proteins but is also less efficient than the use of viral vectors.<sup>25</sup> The expression of a transgene is transient but generally manipulated for up to 8–10 weeks: this method is more beneficial than repeating local injection of growth factors/cytokines.<sup>3,25</sup> To date, the recombinant viral system is well suited for a study of its potential as a therapy of tendon injuries using experimental animal models. Two main strategies for gene transfer using vectors can be envisioned: (i) *in vivo* transfer with a vector that is applied directly to the relevant tissue (*in vivo* direct gene transfer method) and (ii) the removal of cells from the body, transfer of the gene *in vitro*, and then reintroduction into the target site in the body (*ex vivo* indirect gene transfer method).<sup>25</sup> The *in vivo* direct gene transfer method is less invasive and technically easier than transfer of cells *in vitro*. However, the disadvantages of *in vivo* transfer are potential inflammatory responses and non-specific infections at the injury site. The *ex vivo* indirect gene transfer method can ensure the collection of only selected/targeted cells *in vitro* that express the transgene at high concentrations to the injury site with less chance of contamination of viral DNA and proteins. Therefore, the *ex vivo* indirect gene transfer method could be preferable for the treatment of a large and degenerative tendon injury such as that to the rotator cuff, rather than an acute tendon injury.

## **Future directions for tendon injury treatment**

Tendon injuries clinically represent the serious and still unresolved problem of how best to restore a damaged tendon to more nearly normal structural integrity and mechanical strength. Considering the unique feature of tendons with their constant and high mechanical loading, new treatment modalities are required to promote the regeneration of tendon tissues. Tissue engineering offers many approaches for treating tendon injuries and restoring tissues and joint functions. Indeed, a variety of synthetic and biologic scaffolds have been developed. The combination of several scaffolds, including synthetic materials, cells such as MSCs or tendon progenitor cells and growth

factors/cytokines, could be an attractive way to generate an appropriate microenvironment for both transplanted and tendon cells at the injured site. We also foresee many other novel cellular sources and technologies, induced pluripotent stem (iPS) cells or tendon stem/progenitor cells and nanomedicine scaffolds. The emerging interventions using those orthopaedic tissue engineering technologies have shown a therapeutic effect in experimental animal models of tendon injury. However, their efficacy and safety in humans remains to be elucidated in the clinical setting. Unfortunately, to date, the development of new treatment strategies for injured tendons has been hindered because of our limited understanding of basic tendon biology. Nevertheless, the translation of basic research into improved therapeutic approaches is an essential step in the management of patients with tendon injuries, having the potential for significant benefit to public health.

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