CHAPTER 3

Tendon Tissue Engineering

K. Hampson*, N.R. Forsyth, A. El Haj and N. Maffulli

Summary

The successful healing of tendon injuries depends on numerous factors, including anatomical location, vascularity, skeletal maturity and the amount of tissue loss. Although spontaneous healing can occur, this often results in the formation of scar tissue which is morphologically, biochemically and biomechanically different from healthy tendon tissue. This ultimately affects the functionality of the repaired tissue. Tendon tissue engineering aims to induce self-regeneration of the tendon tissue in vivo, or to produce a functional tissue replacement in vitro which can then be implanted into the body. The production of tendon tissue which is both viable and functional requires the generation of a uniaxially orientated matrix. The production and orientation of this matrix can potentially be altered by both biochemical and physical factors, and the combination of these two factors in a dose and time-dependent manner is potentially the key to successfully engineered tendons. This chapter reviews current strategies for tendon tissue engineering and the future challenges associated with this field.

KEYWORDS: Scaffolds, Cell source, Mechanical stimuli, Growth factors, Gene therapy, Tissue engineering

*Correspondence to: K. Hampson, ISTM, Keele University, Staffordshire, UK. E-mail: k.hampson@istm.keele.ac.uk

Topics in Tissue Engineering, Vol. 4. Eds. N Ashammakhi, R Reis, & F Chiellini © 2008.

INTRODUCTION

In the UK the National Health Service (NHS) treats thousands of damaged tendons each year, ranging from repetitive strain injuries (RSIs) to complete ruptures. Tendon injuries are difficult to manage and although spontaneous healing can occur this often results in the formation of scar tissue. Problems arise because the structure of scar tissue differs from healthy tissue which affects both the functionality of the repaired tissue, its movement and its strength [1]. Many tendon injuries occur in athletes and active people and the effect of having tendon tissue with reduced functionality can be devastating to their everyday lives. Current treatments, both conservative and surgical have shown limited success [2], which demonstrates the need for tendon tissue engineering.

STRUCTURE AND FUNCTION

Tendon tissue is a type of connective tissue which physically binds muscles to skeletal structures [3] permitting locomotion and enhancing joint stability [4]. Tendon has a multi-unit hierarchical structure of collagen molecules, fibrils, fibre bundles, fascicles and tendon units [4] designed to resist tensile loads [5]. The organisation of the extracellular matrix (ECM) molecules of tendon at the micrometer and nanometer levels are the principal determinants of the physiological function and the mechanical strength of the tissue [4].

Microscopically, tendon has a crimped, waveform appearance which plays an important role in its mechanical properties [4]. The angle and length of the "crimp pattern" depends on the type of tendon, its anatomical site within the body and its location within the tendon tissue. The differences in the "crimp pattern" affect tendon's mechanical properties, and fibres which have a small crimp angle are mechanically weaker than those with a larger crimp angle [6].

MOLECULAR COMPOSITION

Proteoglycans in the extracellular matrix enhance the mechanically properties of the tissue. Aggrecan helps to retain water within the ECM, increasing the tissues resistance to compression [7]. Decorin is thought to facilitate fibrillar slippage during mechanical deformation [8]. The concentration of proteoglycans varies within the tissue, and depends on the mechanical loading to which the tendon is exposed [9]. The proteoglycan content is higher in the areas which are subjected to compression, given the role of proteoglycans in resisting compression [7, 10].

Tendon tissue contains also glycoproteins, including fibronectin and tenascin-C. Fibronectin, an adhesive glycoprotein located on the surface of collagens [11], is involved the regeneration and repair of tendon [12]. It may also play a role in cell attachment to prevent cell removal due to gliding friction during tendon movement [13]. Tenascin-C is thought to be involved in the ECM network formation which contributes to the mechanical stability of the ECM in tendon tissue by interacting with both the collagen fibrils and the proteoglycan decorin [14]. Tenascin-C is not widely expressed in healthy musculoskeletal tissues, but almost exclusively at the sites subjected to heavy mechanical forces or requiring elastic properties, and is an elastic protein [15]. Tendon tissue also contains elastin, which comprises approximately 2% of the dry weight of tendon tissue [16].

The most abundant molecular component in tendon tissue is collagen type I. It constitutes approximately 60% of the dry mass of the tendon, and about 95% of the total tendon collagen content [17-19]. The remaining 5% of collagens consist mainly of collagens type III and V [4], with collagens type II, VI, IX, X and XI present in trace quantities [20]. Collagen type I molecules self assemble into highly organised fibrils which form collagen fibres [21, 22]. Collagens in the matrix are cross-linked, conferring them a high tensile strength and providing mechanical strength to the tendon tissue [23]. Collagen type III also forms fibrils, but these are smaller and less organised [24].

Although the overall cell content in tendon tissue is low [25], two main types of cells coexist, the tenocytes and the tenoblasts. Both types of these cells have mesenchymal origin. Tenoblasts are immature tendon cells, they are spindle shaped, and have numerous cytoplasmic organelles which reflect their high metabolic activity. These are the predominant cell type in tendon, and often appear in clusters within a localised pericellular region, devoid of collagen fibre anchorage. Tenoblasts mature into tenocytes, which have a fibroblastic morphology and have a much lower nucleus-to-cytoplasm ratio and a lower metabolic activity [26]. Tenocytes are terminally differentiated with a very limited proliferative capacity and are distributed throughout the tissue attached to collagen fibres [27]. Different populations of tenocytes have been identified in tendon tissue, based on their morphologies [28, 29]. However, these cell types have not yet been characterized and their exact function remains unclear [28]. Other types of cells present in tendon tissue are progenitor cells [30], endothelial cells, synovial cells and chondrocytes, although these are much less predominant [31]. There is also a subpopulation of

myofibroblast-like, contractile cells present in normal tendon tissue. They are thought to be involved in the modulation of the contraction-relaxation of the muscle-tendon complex [32].

REQUIREMENT FOR TENDON TISSUE ENGINEERING

There are approximately 2 x 10^5 tendon and ligament repairs performed annually in the U.S. [33]. These can occur through injury and trauma, commonly in the workplace and in sport, but also through overuse and ageing. The most commonly injured tendons are the Achilles and the patellar tendons, with pathology ranging from calcifying tendinopathy, partial tears, to complete ruptures [34, 35]. Injuries which present with pain, swelling, bruising and tearing of the tissue usually occur when the tendon has been under tensile load. Tendons typically affected in this way are the patellar tendon, long head of the biceps, and Achilles tendon. Tendon ruptures can also occur with no episode of a serious injury and little swelling. In these cases, pain and/or inability to play sports will be the major presenting complaint. These injuries often occur in the rotator cuff, extensor carpi radialis brevis or posterior tibial tendon [36]. Tendon injuries are difficult to manage, due to impaired healing, and frequently result in long-term pain and discomfort, which places a chronic burden on health care systems [1].

Successful healing of tendon injuries depends on numerous factors, including anatomical location, vascularity, skeletal maturity, and the amount of tissue loss. Although in most tissues the repair process involves the infiltration of blood cells, mature tendons are poorly vascularised [37-39], and tendon nutrition relies on synovial fluid diffusion rather than vascular perfusion [40]. Although spontaneous healing can occur, this often results in the formation of scar tissue which is morphologically, biochemically and biomechanically different from healthy tendon tissue. This ultimately affects the functionality of the repaired tissue [25]. Severe tendon injuries are difficult to manage because repair rarely results in tissue with fully restored function. Another problem which can occur is the imperfect integrative healing at tendon-tendon or tendon-bone interfaces which can compromise the mechanical properties of the tissue. One reason for this is the formation of fibrous adhesions [19]. Fibrous adhesions occur between the healing tendon and the surrounding tissues, and interfere with tendon gliding. This limits tendon excursion and reduces the functionality of the repaired tendon [41].

The organisation of the collagen fibre crimp pattern determines the mechanical strength of the tissue. Although both normal tissue and tissue formed at a repair site have highly aligned

collagen fibres and a relatively low cell number, morphologically these two types of tissue are quite different. The crimp pattern observed in normal tissue is smaller in length in repaired tendon compared to normal tendon tissue [42]. Also, the diameter of the collagen fibres is significantly smaller in the repaired tissue [43]. These differences in collagen fibril size and alignment result in a tissue which is mechanically weaker and the mechanical properties of the wound repair site are 40-60% of normal tendon levels [44]. These altered structural properties are also thought to contribute to reduced joint stability, degenerative changes, compromised functionality and an increased possibility of re-rupture [45].

CURRENT TREATMENTS

Currently, the management of tendon injury usually follows two routes, conservative or surgical. Conservative management involves rest and pain relief, and can include injection of a variety of drugs, including corticosteroids and physiotherapy. However, given the limited capacity of tendons for self-healing, this type of treatment can result in prolonged treatment times, possible weakness in the affected area, recurrent injury, and partial loss of function [46]. Where natural tissue regeneration does occur, despite remodelling, the biochemical and mechanical properties of the healed tendon may never match those of intact tendon [1]. This often requires extensive and intensive rehabilitation [12].

Mechanical conditioning can be used as a treatment for enhancing tendon healing. Mechanotransduction is the process of a cell converting mechanical stimuli into biochemical signals. Cells able to sense the mechanical signals are described as being mechanosensitive. In theory, all eukaryotic cells are capable of being mechanosensitive. *In vivo*, tendons are exposed to numerous types of strain due to everyday movement. Tendon responds to mechanical forces by adapting its metabolism and structural and mechanical properties [4]. Immobilization of tendon decreases the total weight of the tendon, and reduces its stiffness and tensile strength [47], demonstrating the importance of mechanical forces on tendon homeostasis.

Several factors affect the magnitude of mechanical force applied to tendons during normal locomotion, including the rate and frequency of the applied load [48, 49]. The anatomical location of the tendon in the body also determines what level of mechanical load they are subjected to [50]. This is demonstrated by the Achilles tendon, which withstands higher tensile forces than those of the tibialis anterior [51, 52]. The greater the cross-sectional area of the

muscle attached to the tendon, the higher force it produces and the larger stress a tendon undergoes [53].

Tendons have the ability to adapt to alterations in the mechanical load being applied by changing their structure and composition. The cells in the tendon are responsible for the tendon's adaptive response. These respond to mechanical forces by altering their gene expression patterns, protein synthesis, and cell phenotype [50], which can be used to aid the healing process. However, the duration, frequency, magnitude and type of mechanical stimulation applied to a tendon greatly affect the outcome of the loading regime. Therefore, the amount of loading necessary to improve and/or accelerate the healing process without causing damage to the healing tissue remains unclear [54, 55].

An alternative treatment which is frequently considered in serious injuries is surgical intervention. Surgery can be performed using either percutaneous or open techniques, and, when damage is extensive, tendon grafts may be required [56]. However, these are associated with numerous drawbacks. Artificial implants for tendon repair are relatively successful in reconstructive surgery, but they have a finite lifetime, as their mechanical properties degrade over time. Another drawback with artificial materials are increased inflammatory responses, antigenic reactions, failure at the fixation sites, and lack of long-term biocompatibility [57-59]. One of the most notable drawbacks with synthetic grafts is that they fail to achieve the adaptability demonstrated by functional tendon tissues *in vivo* [2]. Allografts are not widely available, can be expensive and carry the risk of rejection [60]. Autologus grafts are immunologically suitable, but are often associated with some degree of donor morbidity [61].

The management of tendon injuries with either conservative or surgical approaches may have limited success. The equine model has demonstrated the successful repair of tendon tissue with cell therapeutics [62], which suggests a possible route of investigation for tendon tissue engineering.

SCAFFOLDS FOR TENDON TISSUE ENGINEERING

Tissue engineering is an ever expanding field aiming to develop biological substitutes to restore, maintain or improve tissue function [63]. Tissue engineering aims to induce self-regeneration of the tissue *in vivo*, or to produce a functional tissue replacement *in vitro* which can then be implanted into the body [2]. Tendon tissue is a highly organised 3-dimensional (3D) structure.

One strategy for tendon tissue engineering involves combining cells capable of forming tendon with a 3D scaffold to produce a construct which can then implanted into the injury site with the aim that new tissue formation will take place. This strategy relies on an effective cell source and suitable scaffold.

For clinical uses, scaffolds must have specific characteristics because they are exposed to numerous biological and mechanical factors when implanted into the body. Demonstrated biocompatibility of the scaffold material is essential. Scaffolds should not elicit inflammatory response or demonstrate immunogenicity or cytotoxicity [64]. The degradation rate of scaffolds undergoing biodegradation *in vivo* must be compatible with the growth rate of neotissue; by the time the injury site is healed, the scaffold should be totally degraded [65]. The rate of degradation of scaffold material is affected by numerous factors including structure, crystallinity, molecular weight, morphology, porosity and site of implantation [66]. The material must be bioresorbable, so that its by-products are eliminated through natural metabolic pathways in the human body. This results in total elimination of the initial scaffold material with no residual side effects [67]. The scaffold has to have appropriate surface chemistry to enable cellular attachment, proliferation and differentiation, and possess a highly porous structure to permit the diffusion of nutrients throughout the implant [68] and enable tissue integration [69, 70]. *In vitro*, scaffolds should have sufficient mechanical strength to withstand any loading regimes applied, and should also match the mechanical properties of functional tissue when implanted *in vivo* [71].

Scaffolds fall into two general categories, natural and synthetic. Natural scaffolds tested to date include collagens and chitosan [46]. Collagen plays a central role in tendon tissue engineering, because collagen type I is responsible for more than 60% of the dry weight of tendon [2]. Its hierarchical organisation into fibrous bundles provides most of the mechanical strength attributed to tendon tissue. Cell seeded collagen gels have been used in numerous *in vivo* studies [72, 73]. The implantation of a collagen gel seeded with mesenchymal stem cells into an Achilles tendon defect in rabbits improved the biomechanics, structure, and potentially the function of the tendon after injury [74]. However, currently no tenocyte-collagen constructs have been able to achieve sufficient mechanical properties for long term *in vivo* use and the complex architectural structure observed in native tendon tissue is never fully reproduced [2]. Another disadvantage of collagen gels is that collagen contributes to most, but not all, of the mechanical

properties of tendon. Other ECM molecules such as proteoglycans, as described earlier, contribute to the mechanical properties of the overall structure [75] and a scaffold incorporating more of the ECM components of tendon may provide a more promising approach. Collagen hybridised with synthetic polymers has also demonstrated enhanced mechanical properties, and improved the migration of tendon cells [76].

Chitosan-based hyaluronan composite fibre scaffolds have been used to repair rabbit tendons defects. Fibroblast-seeded scaffolds achieved better collagen type I production and mechanical properties after 12 weeks compared to acellular scaffolds and controls, and the tensile strength of the *in situ* fibroblast-seeded scaffolds were significantly greater than non-fibroblast-seeded scaffolds and the controls at 12 weeks [77]. However, this technique was used for repairing rotator cuff injuries in rabbits, and may not be suitable for tendon injuries in humans.

Synthetic biodegradable polymers are often preferentially used due to their reproducible mechanical and physical properties and the control of material impurities. These materials are advantageous because they can be fabricated into various shapes with variable pore frequency and morphology. Several saturated poly-α-hydroxy esters are FDA approved, and the most common polymers used in tissue engineering include polylactide (PLA), polyglycolide (PGA) and poly(lactide-*co*-glycolide) (PLGA) copolymers [64].

PGA fibres seeded with tenocytes have been used to repair 4-cm hen tendon defects. After 14 weeks, histological analysis revealed cell and collagen alignment similar to native collagen and total degradation of the PLGA fibres. The mechanical properties of the repaired tendon after 14 weeks was approximately 80% that of native tendon [78]. However, although this is adequate for some patients, numerous patients with tendon injuries are athletes in whom repaired tendon tissue with sub-optimal functionality is not ideal.

Tissue grafts have also been used as scaffolds. An example is the acellular porcine small intestinal submucosa that was used in ligament replacement in an animal study [79]. The graft was compared to a patellar tendon autograft, and comparable failure forces were found after 12 months. However, the submucosa underwent a dramatic decrease of its biomechanical properties three months after implantation, demonstrating mechanical instability which may not be suitable for use in human tendon repair.

NANOTECHNOLOGY

Nanotechnology can be applied to tendon tissue engineering to produce a biomimetic structures to replicate the native architectural structure of tendon ECM. The ECM of tendon has a highly interconnected porous microstrucutre composed of collagen fibres which are formed hierarchically by nanometer-scale multifibrils. Cells attach and organise themselves around these fibres with diameters smaller than the diameters of the cells. The ECM has a profound influence on tissue regeneration. Biomimetic scaffolds which mimic this structure are a promising approach to tissue engineering [80].

A novel, biodegradable nano-microfibrous polymer scaffold was produced by electrospinning PLGA nanofibers onto a knitted PLGA scaffold to provide a large biomimetic surface for cell attachment. Bone marrow stromal cells seeded onto the novel scaffolds demonstrated increased cell proliferation and higher expression of collagen I, decorin, and biglycan genes compared to co-knitted PLGA scaffolds [81].

CELL SOURCE

There are different opinions on what is the best type of cell to use for tendon tissue engineering. Short term studies in rabbits have demonstrated that there are numerous sources of tendon progenitor cells within the body. Kryger *et al.* [82] compared tenocytes, bone marrow derived mesenchymal stem cells, adipose tissue-derived mesenchymal stem cells, and tendon sheath fibroblasts. Each cell type was seeded into acellularised tendon tissue and implanted *in vivo* into a flexor tendon defect. Histological analysis six weeks after implantation revealed viable cells within the architecture of the tendon for all four cell types. It is unclear, however, which cell type resulted in the most mechanically stable tissue, a most important property of the regenerated tissue. In principle, the primary sources of human cells for therapeutic repair are autologous or allogenic tenocytes, autologous or allogenic adult stem cells, cord blood stem cells (CBSCs) or embryonic stem cells (ESCs) [46].

The use of autologous or allogenic tendon cells can be very problematic. Donor site morbidity is an obvious problem. Another problem is the low number of cells obtained from the explanted tissue because tendons are relatively acellular, containing few tenocytes [46]. The isolation of low cell numbers requires the extended proliferation of tendon cells prior to implantation *in vivo*. Tenocytes do not proliferate for long during *in vitro* culture systems, and

with increasing passage number there is a decrease in cell density. During this period, tenocytes de-differentiate and lose their characteristic tenocyte morphology [83]. This can affect the resultant cell phenotype. The expansion protocol necessary to achieve an appropriate cell number is lengthy, thus increasing the patients waiting time [2]. However, recent studies by Bi *et al.* [30] isolated a unique cell population in individuals aged between 8 and 12 years old which they termed 'tendon stem/progenitor cells'. These were shown to be multipotent and may represent a promising cell source for tendon tissue engineering strategies [30].

Adult stem cells, cord blood cells or ESCs all vary in their ability to form different cell lineages. The hierarchy runs from the pluripotent ESCs to the potentially pluripotent CBSCs to the multipotent MSCs [46]. Although much research has been carried out involving the differentiation of CBSCs and ESCs along numerous lineages, there is little evidence of the *in vitro* capacity of these two cell types to differentiate into either tenocytes or tendons. Scleraxis (Scx) is a transcription factor, specifically expressed in tendons and ligaments, and is involved in the activation of the proα1(1) collagen gene in tendon fibroblasts [23]. The isolation of *Scleraxis*-expressing progenitor cells from partially differentiated ESC represents is a promising approach for generating tendon lineage cells [46].

Adult MSCs have been shown to be tendon-capable. However, these descriptions originate almost entirely from animal model studies involving rat, rabbit and horse [5, 84, 85]. Autologous MSC therapeutics can enhance tendon tissue repair in a horse model, but at present the methodologies for the differentiation of human MSCs into tenocytes or tendon tissue remain unclear and unproven [46]. Despite the lack of evidence, MSCs have a broadly assumed capacity to differentiate along the tenocyte lineage [86, 87]. Although horse tendon injuries are already being treated with autologous bone marrow-derived MSCs [62], studies with rabbits have demonstrated that MSCs alone may not be adequate to regenerate fully functional tendon tissue. Awad *et al.* [84] implanted autologous adult MSCs into a surgically induced patellar tendon defect in rabbits. This improved some of the biomechanical properties of the lesion, but did not improve the tendon's microstructure.

Although there is a potential for all of the discussed cell types to form tendon-capable cells, or tendon itself, the lack of data makes conclusions regarding the most suitable source of cells for tendon repair difficult. More research is required with respect to the optimal conditions for the *in vitro* expansion and differentiation of tendon cells and their progenitors [46]. Gene

therapies, genetically modifying the MSCs prior to implantation, may present a possible improvement to the success of these strategies and will be discussed later.

ENHANCING TENDOGENESIS

Tendon cells are capable of mechanotransduction, and the application of mechanical forces to tendons and tendon cells *in vitro* has been shown to have positive effects. Skutek *et al.* [88] demonstrated that application of 5% stretch, at a frequency of 1 Hz to human tendon fibroblasts increases the secretion of the transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF). The increases in expression as a result of the loading regime were dependent on the number of loading cycles, again demonstrating that the type of loading regime used is crucial to achieve a positive effect. Mechanical stretch also influences human tendon fibroblast proliferation [55].

Tendon cells also respond to mechanical forces by orientating themselves in the direction of the applied force. The application of cyclic tension to lacerated chicken tendon *in vitro* for 14 days resulted in an increase in the production of newly proliferated fibroblasts, aligned in the direction of tension and the tissue was thicker than that seen in the non-tension group at the same time interval. Mechanical stretching also increased the production of collagen [89]. An increase in the production of collagen has also been demonstrated by Noth *et al.* [90], who applied cyclic stretch to MSCs seeded in a collagen type I matrix for 8 hours per day. This increased the gene expression of collagen types I and III, fibronection and elastin compared to non-stretched controls.

Numerous strategies for the enhancement of tendogenesis have used chemical stimulation. A number of standard mesenchymal lineage differentiation protocols rely on the inclusion of glucocorticoids in the culture media. Dexamethasone is a synthetic glucocorticoid which has been used in both osteogenic, chondrogenic and adipogenic protocols [91-93]. However, the administration of dexamethasone to tenocyte cultures *in vitro* can reduce the number of viable cells and suppress cell proliferation. It can also reduce the amount of collagen synthesis [94]. Dexamethasone can also suppress proteoglycan production in cultured human tenocytes [95].

Bone morphogenetic proteins (BMPs) are a family of highly related molecules which are members of the TGF-β superfamily [96]. Individually, the members of this subfamily of secreted

molecules are termed either BMPs, osteogenic proteins (OPs), cartilage-derived morphogenetic proteins (CDMPs), or growth and differentiation factors (GDFs). Most BMPs have the ability to induce bone and cartilage formation in animals by inducing the differentiation of mesenchymal progenitor cells along the cartilage or bone lineage [97]. However, there have been numerous experiments demonstrating that the GDFs 5/6 and 7 can improve tendon healing and repair [3, 98].

The role of the human homologue of GDF–7 (BMP-12) on the proliferation and differentiation of human tenocytes has been assessed [94]. The addition of recombinant human BMP-12 to human patellar tendon fibroblasts increased their proliferation and the expression of procollagen types I and III. Immunohistological staining of healthy human patellar samples also demonstrated that BMP-12 is present in the sites of active cell proliferation and procollagen type I production which suggests that BMP-12 may play a role in the early stages of tendon regeneration. BMP-12 may also increase the mechanical properties of repaired tendon tissue by regulating the deposition of the ECM [94]. BMP-13 increases the proliferation of human patellar fibroblasts and the gene expression of pro-collagen type I [95]. BMP-2 enhances collagen production in canine tenocytes [99], and can improve tendon-bone integration in canine disease models [100].

bFGF and PDGF are the major mitogenic agents for fibroblasts [2]. The addition of these growth factors to rat tenocytes has been shown to increase their cell proliferation *in vitro* [101]. This effect is maximized when these growth factors are used in combination. PDGF has been shown to reverse the effects of glucocorticoid injections, which can reduce tendon cell viability, cell proliferation and collagen synthesis [94]. The addition of bFGF to a patellar tendon gap wound healing model in rats increased the cell proliferation [102] and collagen type III production in a dose dependent manner [103].

Growth factors injected directly into the wound site enhance tendon repair. The injection of bFGF into rat patellar tendon injury resulted in an increase in cell proliferation and collagen type III production [103]. Interestingly, rather than increase the up-regulation of a particular growth factor, some strategies are based on the reduction of a particular growth factor in the injury site. High levels of TGF-β1 are associated with adhesion formation in tendons which can affect the functionality of the tissue. During the repair process, inflammatory cells around the injured tendon release large numbers of cytokines and growth factors, including TGF-β which

promote cellular proliferation, migration and adhesion. Animal studies have demonstrated that neutralizing the effects of TGF-β can prevent adhesion formation [104, 105].

GENE THERAPY

Gene therapy-based tendon tissue engineering is an attractive new approach to the management of tendon injuries. For effective tissue regeneration it is important to deliver genes which can aid in the healing of the tissue to the site of injury. There are two possible strategies for this. The first one is the *in vivo* transfer of the gene within a vector which is applied directly to the target tissue. Lou *et al*. [106] injected BMP-12 into a complete tendon laceration chicken model. This resulted in a 2-fold increase in the tensile strength and stiffness of the repaired tendon. The gene encoding human BMP-14 has been directly applied to the transected and repaired Achilles tendons of Sprague-Dawley rats. A sham control group received no viral-mediated infection after repair. Tendons transduced with the BMP-14 gene exhibited less visible gapping, a greater number of neotenocytes at the site of healing, and a 70% increase in tensile strength compared to the control tendons two weeks after repair [107]. Insulin-like growth factor I (IGF-I) also increases the rate of healing in a transected Achilles tendon in rats. Postoperatively, the rats whose tendon injuries received this growth factor had a significantly decreased time to functional recovery than rats in the untreated groups, thought to be due to the anti-inflammatory effect of IGF-I [108].

One major disadvantage with the direct *in vivo* transfer strategy is the possibility of transfecting cells adjacent to the target tissue. This risk is further increased by the low cell concentration in tendon tissue. To achieve maximum transfection efficiency, the vector would have to have high transgenic activity [109]. Another problem with this approach is the possibility of an immune response to the vector which can result in lymphocytic infiltration [80].

In the second strategy, cells are harvested from the body, transfected with the target gene *in vitro* and, after culture, reintroduced into the target site *in vivo.* This strategy involves a greater margin of safety, because modified cells can be tested *in vitro* before administration, and the viral DNA is not administered directly to the host tissue. This represents a more promising approach for *in vivo* human use [80]. Smads are a group of related intracellular proteins that transmit TFG-β superfamily signals from ligand-activated cell surface receptors to the nucleus [19]. Smad-8 is a receptor-regulated Smad (R-Smad) associated with BMP signalling [5].

Hoffman *et al.* [5] transfected the Smad-8 gene into an MSC line which co-expressed BMP-2. When ectopically implanted in mice, the cells differentiated to form tenocytes and resulted in the regeneration of an Achilles tendon partial defect in rats when implanted *in vivo*. Histological analysis demonstrated the formation of dense connective tissue with extensive formation of collagen fibres characteristic of tendon.

One of the most important uses of gene transfer in the field of tendon tissue engineering lies, perhaps, in the study of the effects of specific gene expression within mature tendon and progenitor cells. This can lead to a better understanding of the pattern of gene expression within tendon and ultimately the regeneration process. The transfer of PDGF-B DNA into tenocytes increased the expression of collagen type I, suggesting that PDGF is involved in the regulation of collagen type I in tendons, and demonstrating the benefits of this molecular technique [110]. MSCs transfected with the BMP-12 gene became elongated, and their processes became thinner and were interwoven into a network. There were also more organelles in the transfected cells than in the unaltered MSCs. Transfected cells expressed BMP-12, collagen type I and scleraxis, but failed to express collagen type III. This research demonstrated that MSCs can be introduced to differentiate into tenocytes by BMP-12 gene transfection, and bone marrow MSCs may serve as a suitable seed cell for applications in tendon tissue engineering [50].

CONCLUDING REMARKS

Although spontaneous tendon healing can occur, this often results in neotissue with reduced functionality. Both allografts and autografts have limited success and availability, and synthetic grafts fail to meet the long-term stability and mechanical requirements for a successful tendon replacement. Clearly, another approach is needed to produce functional tissue with biochemical and biomechanical properties comparable to native tendon tissue. Currently, the majority of tissue engineered substitutes for tendon have failed to achieve appropriate mechanical properties required for optimal function *in vivo*. Tendon tissue is exposed to both biochemical and mechanical stimuli *in vitro*, and a strategy which combines a suitable tendon progenitor cell, with an appropriate scaffold with or without the incorporation of a growth factor and mechanical stimulation, may provide a tendon substitute with biochemical and mechanical properties more suitable for *in vivo* implantation. However, the lack of evidence for the most effective scaffold material, cell type, loading regime or *in vitro* culture conditions for the production of a

successful tendon substitute determines that the main goals for present tendon tissue engineering strategies must be to define the optimal parameters for each of these research areas within the field of tendon tissue engineering.

References

1. Sharma, P. and N. Maffulli, *Tendon Injury and Tendinopathy: Healing and Repair.* J Bone Joint Surg Am, 2005. **87**(1): p. 187-202.
2. Bagnaninchi, P., et al., *Tissue engineering for tendon repair.* Br J Sports Med, 2006: p. bjsm.2006.030643.
3. Wolfman, N., et al., *Ectopic Induction of Tendon and Ligament in Rats by Growth and Differentiation Factors 5, 6, and 7, Members of the TGF-beta Gene Family.* Journal Clinical Investigation, 1997. **100**(2): p. 321-330.
4. Wang, J.H.C., *Mechanobiology of tendon.* Journal of Biomechanics, 2006. **39**(9): p. 1563-1582.
5. Hoffmann, A. and G. Gross, *Tendon and ligament engineering in the adult organism: mesenchymal stem cells and gene-therapeutic approaches.* International Orthopaedics, 2007.
6. Wilmink, J., A. Wilson, and A. Goodship, *Functional significance of the morphology and micromechanics of collagen fibres in relation to partial rupture of the superficial digital flexor tendon in racehorses.* Research in Veterinary Science, 1992. **53**: p. 354–359.
7. Vogel, K. and T. Koob, *Structural specialization in tendons under compression.* International Review of Cytology, 1989. **115**: p. 267-293.
8. Pins, G., et al., *Self-assembly of collagen fibers. Influence of fibrillar alignment and decorin on mechanical properties.* Biophysical Journa, 1997. **73**: p. 2164-2172.
9. Berenson, M., et al., *Proteoglycans of human rotator cuff tendons.* Journal of Orthopaedic Research, 1996. **14**(4): p. 518-525.
10. Koob, T. and K. Vogel, *Site-related variations in glycosaminoglycan content and swelling properties of bovine flexor tendon.* Journal of Orthopaedic Research, 1987. **5**(3): p. 414-424.
11. Lawler, J., *The structural and functional properties of thrombospondin.* Blood, 1986. **67**(5): p. 1197-1209.
12. Sharma, P. and N. Maffulli, *Biology of tendon injury: healing, modeling and remodeling.* Journal of musculoskeletal & neuronal interactions, 2006. **6**(2): p. 181-190.
13. Banes, A. and G.W.L.A.G.B.H.D.P.Y.G.D.B.S.W.L. Dahners, *Tendon synovial cells secrete fibronectin in vivo and in vitro.* Journal of Orthopaedic Research, 1988. **6**(1): p. 73-82.
14. Elefteriou, F., et al., *Binding of tenascin-X to decorin.* FEBS Letters, 2001. **495**(1-2): p. 44-47.
15. Kannus, P., et al., *Location and Distribution of Non-collagenous Matrix Proteins in Musculoskeletal Tissues of Rat.* The Histochemical Journal, 1998. **30**(11): p. 799-810.
16. Jozsa, L., et al., *Alterations in dry mass content of collagen fibers in degenerative tendinopathy and tendon-rupture.* Journal of Biomechanics, 1989. **9**: p. 140-146.

17. Evans, J. and J. Barbenel, *Structural and mechanical properties of tendon related to function.* Equine Veterinary Journal, 1975. **7**(1): p. 1-8.
18. Fenwick, S., B. Hazleman, and G. Riley, *The vasculature and its role in the damaged and healing tendon.* Arthritis Research, 2002. **4**(4): p. 252-260.
19. Towler, D. and R. Gelberman, *The alchemy of tendon repair: a primer for the (S)mad scientist.* Journal of Clinical Investigation, 2006. **116**(4): p. 863-866.
20. Fukuta, S., et al., *Identification of types II, IX and X collagens at the insertion site of the bovine achilles tendon.* Matrix Biology, 1998. **17**(1): p. 65-73.
21. Bailey, A. and N. Light, *Intermolecular cross-linking in fibrotic collagen.* Ciba Foundation symposium 1985. **114**: p. 80-96.
22. Eyre, D., M. Paz, and P. Gallop, *Cross-linking in collagen and elastin.* Annual Review of Biochemistry 53, 1984. **53**: p. 717-748.
23. Lejard, V., et al., *Scleraxis and NFATc Regulate the Expression of the Pro-{alpha}1(I) Collagen Gene in Tendon Fibroblasts.* Journal Biological Chemistry, 2007. **282**(24): p. 17665-17675.
24. Lapiere, C., B. Nusgens, and G. Pierard, *Interaction between collagen type I and type III in conditioning bundles organization.* Connective Tissue Research, 1977. **5**: p. 21-29.
25. Le Huec, J., et al., Epicondylitis after treatment with fluoroquinolone antibiotics. Journal of Bone and Joint Surgery, 1995. 77(B): p. 293-295.
26. Kannus, P., L. Jozsa, and M. Jarvinnen, *Basic science of tendons*, in *Principles and practice of orthopaedic sports medicine*, W.J. Garrett, K. Speer, and D. Kirkendall, Editors. 2000, Lippincott Williams and Wilkins: Philadelphia 2000. p. 21-37.
27. Chuen, F., et al., *Immunohistochemical Characterization of Cells in Adult Human Patellar Tendons.* Journal of Histocheistry and Cytochemistry, 2004. **52**(9): p. 1151-1157.
28. Goodship, A., H. Birch, and A. Wilson, *The pathobiology and repair of tendon and ligament injury.* Veterinary Clinics of North America: Equine Practise, 1994. **10**(2): p. 323-349.
29. Smith, R. and P. Webbon, *The physiology of normal tendon and ligament.* Proceedings 1st Dubai Equine International Symposium, 1996: p. 55-81.
30. Bi, Y., et al., *Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche.* Nature Medicine, 2007. **advanced online publication**.
31. McNeilly, C., et al., *Tendon cells in vivo form a three dimensional network of cell processes linked by gap junctions.* Journal of Anatomy, 1996. **189**(3): p. 593-600.
32. Ippolito, E., et al., *Ultrastructural and immunochemical evidence of actin in the tendon cells.* Clinical Orthopaedics, 1977. **126**: p. 282-284.
33. Pennisi, *Tending Tender Tendons.* Science, 2002. **295**(5557): p. 1011.
34. Jarvinen, T., et al., *Achilles Tendon Disorders: Etiology and Epidemiology.* Foot and Ankle Clinics of North America, 2005. **10**(2): p. 255-266.
35. Sheng, P., et al., *Revision total knee arthroplasty with the Total Condylar III system: A comparative analysis of 71 consecutive cases of osteoarthritis or inflammatory arthritis.* Acta Orthopaedica, 2006. **77**(3): p. 512 - 518.
36. Kibler, W.B., *Diagnosis, treatment and rehabilitation principles in complete tendon ruptures in sports.* Scandinavian Journal of Medicine & Science in Sports, 1997. **7**(2): p. 119-129.

37. Takasugi, H., et al., *Three-dimensional architecture of blood vessels of tendons demonstrated by corrosion casts.* Hand, 1978. **10**(1): p. 9-15.
38. Schmidt-Rohlfing, B., et al., *The blood supply of the Achilles tendon.* International orthopaedics, 1992. **16**(1): p. 29-31.
39. Ahmed, I. and M.L.P.M.R.W.S.G.K. Sefton, *Blood supply of the achilles tendon.* Journal of Orthopaedic Research, 1998. **16**(5): p. 591-596.
40. Potenza, A., *Tendon healing within the flexor digital sheath in the dog.* The Journal of bone and joint surgery. American volume, 1962. **44**(A): p. 49-64.
41. Meyers, S., et al., *Effect of hyaluronic acid/chondroitin sulfate on healing of full-thickness tendon lacerations in rabbits.* Journal of Orthopaedic Research, 1989. **7**(5): p. 683-689.
42. Miyashita, H., M. Ochi, and Y. Ikuta, *Histological and biomechanical observations of the rabbit patellar tendon after removal of its central one-third.* Archives of Orthopaedic and Trauma Surgery, 1997. **116**(8): p. 454-462.
43. Järvinen, T. and T.L.N.J.P.K.L.J.M. Järvinen, *Collagen fibres of the spontaneously ruptured human tendons display decreased thickness and crimp angle.* Journal of Orthopaedic Research, 2004. **22**(6): p. 1303-1309.
44. Buck, R.C., *Regeneration of tendon.* The Journal of Pathology and Bacteriology, 1953. **66**(1): p. 1-18.
45. Hankemeier, S., et al., *Tissue engineering of tendons and ligaments by human bone marrow stromal cells in a liquid fibrin matrix in immunodeficient rats: Results of a histologic study.* Archives of Orthopaedic and Trauma Surgery, 2007.
46. Bullough, R., et al., Disability and Rehabilitation, in press.
47. Woo, S., et al., *Mechanical properties of tendons and ligaments. II. The relationships of immobilization and exercise on tissue remodeling.* Biorheology, 1982. **19**(9): p. 397-408.
48. Finni, T., P.V. Komi, and J. Lukkariniemi, *Achilles tendon loading during walking: application of a novel optic fiber technique.* European Journal of Applied Physiology, 1998. **77**(3): p. 289-291.
49. Kyröläinen, H., et al., *Neuromuscular Behaviour of the Triceps Surae Muscle-Tendon Complex during Running and Jumping.* International Journal of Sports Medicine, 2003(3): p. 153-155.
50. Wang, Q., Z. Chen, and Y. Piao, *Mesenchymal Stem Cells Differentiate into Tenocytes by Bone Morphogenetic Protein (BMP) 12 Gene Transfer.* Journal of Bioscience and Bioengineering, 2005(4): p. 418-422.
51. Maganaris, C.N., *Tensile properties of in vivo human tendinous tissue.* Journal of Biomechanics, 2002. **35**(8): p. 1019-1027.
52. Maganaris, C.N. and J.P. Paul, *Tensile properties of the in vivo human gastrocnemius tendon.* Journal of Biomechanics, 2002. **35**(12): p. 1639-1646.
53. Kellis, E., *Quantification of Quadriceps and Hamstring Antagonist Activity.* Sports Medicine, 1998. **25**(1): p. 37-62.
54. Steadman, J., R. Forster, and J. Silferskiold, *Rehabilitation of the knee.* Clinical Sports Medicine, 1989. **8**: p. 605-627.
55. Zeichen, J., M. van Griensven, and U. Bosch, *The Proliferative Response of Isolated Human Tendon Fibroblasts to Cyclic Biaxial Mechanical Strain.* Am J Sports Med, 2000. **28**(6): p. 888-892.

56. Miller, D. and R.J. Waterston S, Barrass V, Maffulli N, *Conservative management, percutaneous or open repair of acute Achilles tendon rupture: a retrospective study.* Scottish Medical Journal, 2005. **50**(4): p. 160-165.
57. Bader, K. and J. Curton, *A successful silicone tendon prosthesis.* Archives of Surgery, 1968. **97**(3): p. 406-411.
58. Mahoney, J., L. Farkas, and W. Lindsay, *Quality of tendon graft healing in silastic pseudosheaths: breaking-strength studies.* Surgical Forum, 1976. **27**(62): p. 572-573.
59. Beris, A., et al., *Two-stage flexor tendon reconstruction in zone II using a silicone rod and a pedicled intrasynovial graft.* The Journal of Hand Surgery, 2003. **28**(4): p. 652-660.
60. Kim, C. and R. Pedowitz, *Part A:graft choices and the biology of graft healing*, in *Daniel's Knee Injuires*

R. Pedowitz, J. O'Conner, and W. KAkeson, Editors. 2007, Lippincott Williams & Wilkins: Philadelphia. p. 435-491.

61. Wang, S., et al., *Infections and human tissue transplants: review of FDA MedWatch reports 2001-2004.* Cell Tissue Bank, 2007.
62. Giovannini, S., et al., *Multilineage differentiation potential of equine blood-derived fibroblast-like cells.* Differentiation, 2007. **epub**.
63. Nerem, R., *Cellular engineering.* Annals of biomedical engineering, 1991. **19**(5): p. 529-545.
64. Rezwan, K., et al., *Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering.* Biomaterials, 2006. **27**(18): p. 3413-3431.
65. Salgado, A., O. Coutinho, and R. Reis, *Bone Tissue Engineering: State of the Art and Future Trends.* Macromolecular Bioscience, 2004. **4**(8): p. 743-765.
66. Gunatillake, P. and R. Adhikari, *Biodegradable synthetic polymers for tissue engineering.* European Cells and Materials, 2003. **5**: p. 1-16.
67. Vert, M., et al., *Bioresorbability and biocompatibility of aliphatic polyesters.* Journal of Materials Science: Materials in Medicine, 1992. **3**(6): p. 432-446.
68. Yang, S., et al., *The Design of Scaffolds for Use in Tissue Engineering. Part I. Traditional Factors.* Tissue Engineering, 2001. **7**(6): p. 679-689.
69. Levenberg, S. and R. Langer, *Advances in tissue engineering.* Current Topics in Developmental Biology, 2004. **61**: p. 113-134.
70. Griffith, L., *Emerging Design Principles in Biomaterials and Scaffolds for Tissue Engineering.* Annals of New York Academy Sciences, 2002. **961**(1): p. 83-95.
71. Sachlos, E. and J. Czernuszka, *Making Tissue Engineering Scaffolds Work. Review: The application of solid freeform fabrication technology to the production of tissue engineering scaffolds* European Cells and Materials, 2003. **5**: p. 29-40.
72. Takakuda, K. and H. Miyairi, *Tensile behaviour of fibroblasts cultured in collagen gel.* Biomaterials, 1996. **17**: p. 1393-1397.
73. Grinnell, F., *Fibroblast biology in three-dimensional collagen matrices.* Trends in Cell Biology, 2003. **13**: p. 264-269.
74. Young, R. and D.L.B.W.W.A.I.C.S.L.G.D.J. Fink, *Use of mesenchymal stem cells in a collagen matrix for achilles tendon repair.* Journal of Orthopaedic Research, 1998. **16**(4): p. 406-413.

75. Canty, E. and K.E. Kadler, *Collagen fibril biosynthesis in tendon: a review and recent insights.* Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 2002. **133**: p. 979-985.
76. Ide, A., et al., *Collagen hybridization with poly(l-lactic acid) braid promotes ligament cell migration.* Materials Science & Engineering C, 2001. **17**(1): p. 95-99.
77. Funakoshi, T., et al., *Application of Tissue Engineering Techniques for Rotator Cuff Regeneration Using a Chitosan-Based Hyaluronan Hybrid Fiber Scaffold.* Am J Sports Med, 2005. **33**(8): p. 1193-1201.
78. Cao, Y., et al., *Bridging Tendon Defects Using Autologous Tenocyte Engineered Tendon in a Hen Model.* Plastic & Reconstructive Surgery, 2002. **110**(5): p. 1280-1289.
79. Badylak, S., et al., *Naturally Occurring Extracellular Matrix as a Scaffold for Musculoskeletal Repair.* Clinical Orthopaedics & Related Research, 1999. **367**(Supplement): p. S333-343.
80. Huang, D., G. Balian, and A. Chhabra, *Tendon Tissue Engineering and Gene Transfer: The Future of Surgical Treatment.* The Journal of Hand Surgery, 2006. **31**(5): p. 693-704.
81. Sahoo, S., et al., *Characterization of a Novel Polymeric Scaffold for Potential Application in Tendon/Ligament Tissue Engineering.* Tissue Engineering, 2006. **12**(1): p. 91-99.
82. Kryger, G., et al., *A Comparison of Tenocytes and Mesenchymal Stem Cells for Use in Flexor Tendon Tissue Engineering.* The Journal of Hand Surgery, 2007. **32**(5): p. 597-605.
83. Yao, L., et al., *Phenotypic Drift in Human Tenocyte Culture.* Tissue Engineering, 2006. **12**(7): p. 1843-1849.
84. Awad, H., et al., *Autologous Mesenchymal Stem Cell-Mediated Repair of Tendon.* Tissue Engineering, 1999. **5**(3): p. 267-277.
85. Smith, R., et al., *Isolation and implantation of autologous equine mesenchymal stem cells from bone marrow into the superficial digital flexor tendon as a potential novel treatment.* Equine veterinary journal, 2003. **35**(1): p. 99-102.
86. Pittenger, M.F., et al., *Multilineage Potential of Adult Human Mesenchymal Stem Cells.* Science, 1999. **284**(5411): p. 143-147.
87. Caplan, A. and S.P. Bruder, *Mesenchymal stem cells: building blocks for molecular medicine in the 21st century.* Trends in Molecular Medicine, 2001. **7**(6): p. 259-264.
88. Skutek, M., et al., *Cyclic mechanical stretching modulates secretion pattern of growth factors in human tendon fibroblasts.* European Journal of Applied Physiology, 2001. **86**(1): p. 48-52.
89. Tanaka, H., et al., *Effect of cyclic tension on lacerated flexor tendons in vitro.* The Journal of hand surgery, 1995. **20**(3): p. 467-473.
90. Nöth, U., et al., *Anterior cruciate ligament constructs fabricated from human mesenchymal stem cells in a collagen type I hydrogel.* Cytotherapy, 2005. **7**(5): p. 447 - 455.
91. Cheng, S.L., et al., *Differentiation of human bone marrow osteogenic stromal cells in vitro: induction of the osteoblast phenotype by dexamethasone.* Endocrinology, 1994. **134**(1): p. 277-286.
92. Cui, Q., G.J. Wang, and G. Balian, *Steroid-Induced Adipogenesis in a Pluripotential Cell Line from Bone Marrow.* J Bone Joint Surg Am, 1997. **79**(7): p. 1054-63.

93. Derfoul, A., et al., *Glucocorticoids Promote Chondrogenic Differentiation of Adult Human Mesenchymal Stem Cells by Enhancing Expression of Cartilage Extracellular Matrix Genes.* Stem Cells, 2006. **24**(6): p. 1487-1495.
94. Wong, M.W.N., et al., *Effect of Dexamethasone on Cultured Human Tenocytes and Its Reversibility by Platelet-Derived Growth Factor.* J Bone Joint Surg Am, 2003. **85**(10): p. 1914-1920.
95. Wong, M.W.N., et al., *Glucocorticoids suppress proteoglycan production by human tenocytes.* Acta Orthopaedica, 2005. **76**(6): p. 927 - 931.
96. Wozney, J., et al., *Novel regulators of bone formation: molecular clones and activities.* Science, 1988. **242**(4885): p. 1528-1534.
97. Ducy, P. and G. Karsenty, *The family of bone morphogenetic proteins.* Kidney International, 2000. **57**: p. 2207-2214.
98. Forslund, C. and P. Aspenberg, *Improved Healing of Transected Rabbit Achilles Tendon after a Single Injection of Cartilage-Derived Morphogenetic Protein-2.* American Journal of Sports Medicine, 2003. **31**(4): p. 555-559.
99. Thomopoulos, S., et al., *Effect of Several Growth Factors on Canine Flexor Tendon Fibroblast Proliferation and Collagen Synthesis In Vitro.* The Journal of Hand Surgery, 2005. **30**(3): p. 441-447.
100. Martinek, V., et al., *Enhancement of Tendon-Bone Integration of Anterior Cruciate Ligament Grafts with Bone Morphogenetic Protein-2 Gene Transfer : A Histological and Biomechanical Study.* J Bone Joint Surg Am, 2002. **84**(7): p. 1123-1131.
101. Stein, L., *Effects of serum, fibroblast growth factor, and platelet-derived growth factor on explants of rat tail tendon: a morphological study.* Acta anatomica, 1985. **123**(4): p. 247-252.
102. Chan, B.P.B.C., Kai Ming; Maffulli, Nicola; Webb, Sarah; Lee, Kenneth K.H, *Effect of Basic Fibroblast Growth Factor; An In Vitro Study of Tendon Healing.* Clinical Orthopaedics & Related Research. , 1997. **342**(239-247).
103. Chan, B.P., et al., *Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: A rat patellar tendon model.* Acta Orthopaedica, 2000. **71**(5): p. 513 - 518.
104. Jorgensen, H., et al., *Neutralisation of TGF[beta] or binding of VLA-4 to fibronectin prevents rat tendon adhesion following transection.* Cytokine, 2005. **30**(4): p. 195-202.
105. Costa, M.A., et al., *Tissue Engineering of Flexor Tendons: Optimization of Tenocyte Proliferation Using Growth Factor Supplementation.* Tissue Engineering, 2006. **12**(7): p. 1937-1943.
106. Lou, J. and Y.T.M.B.M.J.S.P. Manske, *BMP-12 gene transfer augmentation of lacerated tendon repair.* Journal of Orthopaedic Research, 2001. **19**(6): p. 1199-1202.
107. Bolt, P., et al., *BMP-14 Gene Therapy Increases Tendon Tensile Strength in a Rat Model of Achilles Tendon Injury.* Journal of Bone and Joint Surgery American, 2007. **89**(6): p. 1315-1320.
108. Kurtz, C., et al., *Insulin-Like Growth Factor I Accelerates Functional Recovery from Achilles Tendon Injury in a Rat Model.* American Journal of Sports Medicine, 1999. **27**(3): p. 363-369.
109. Bonadio, J., *Tissue engineering via local gene delivery:: Update and future prospects for enhancing the technology.* Advanced Drug Delivery Reviews, 2000. **44**(2-3): p. 185-194.

110. Wang, X., P. Liu, and J. Tang, *Tendon healing in vitro: Genetic modification of tenocytes with exogenous PDGF gene and promotion of collagen gene expression.* The Journal of Hand Surgery, 2004. **29**(5): p. 884-890.