A MINI REVIEW ON THE BASIC KNOWLEDGE ON TENDON: REVISITING THE NORMAL & INJURED TENDON

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ABSTRACT

Tendon is a dense connective tissue that connects muscle to bone. Tendon can adapt to mechanical forces passing across it, through a reciprocal relationship between its cellular components (tenocytes and tenoblasts) and the extracellular matrix (ECM). In early development, the formation of scleraxis-expressing tendon progenitor population in the sclerotome is induced by a fibroblast growth factor signal secreted by the myotome. Tendon injury has been defined as a loss of cells or ECM caused by trauma. It represents a failure of cells and matrix adaptation to mechanical loading. Injury initiates attempts of tendon to repair itself, which has been defined as replacement of damaged or lost cells and ECM by new cells or new matrices. Tendon healing generally consists of four different phases: the inflammatory, proliferation, differentiation and remodelling phases. Clinically, tendons are repaired with a variety of surgical techniques, which show various degrees of success. In order to improve the conventional tendon repair methods, current tendon tissue engineering aims to investigate a repair method which can restore tissue defects with living cells, or cell based therapy. Advances in tissue engineering techniques would potentially yield to a cell-based product that could regenerate functional tendon tissue.

Keywords: *Cell based therapy, cell differentiation, expression profile, orthopaedics, stem cell biology, tendon tissue engineering*

STRUCTURE AND FUNCTION OF NORMAL TENDON

A. Tendon, Tenocyte and Tendon Extracellular Matrix

Tendon is dense connective tissue which connects muscle to bone and allows transmission of forces generated by muscle to bone, resulting in joint movement. It is living tissue with mechanical adaptation ability that allows it to respond to mechanical forces (eg. high tensional loading). This is achieved through changes in the metabolism as well as its structural and mechanical properties (1-3). These critical biological and biomechanical roles of tendon are played through a reciprocal relationship between its two main components, i.e. cells and extracellular matrix (ECM) (Table 1).

The overall cell content in tendon tissue is low (20%). Tenocytes and tenoblasts are the two main cell types which coexist in tendon. Both of these cells are of mesenchymal origin and they constitute about 90-95% of the cellular component of tendons (4). Tenoblasts are immature tendon cells. They are spindle-shaped and have numerous cytoplasmic organelles. The high organelle content reflects their high metabolic activity. As they mature, tenoblasts become elongated and transformed into tenocytes. Tenocytes have lower nucleus-to-cytoplasm ratio than tenoblasts. These cells lie between the collagen fibers along

Table 1: Structural compositions of tendon (1,7).

Component	Total (%)
I. Cellular materials	**20**
i. Tenocytes and tenoblasts	90-95
ii. Others (Chondrocytes, synovial cells and vascular cells)	5-10
II. Extracellular matrix (ECM)	**80**
i. Water	60-80
ii. Dry mass	20-40
a. Collagen	75-85
Type-I	95-99
Type-III and V	1-5
Others (Type II, VI, IX, X and XI)	Trace amount
b. Ground substance (Proteoglycan, glycoproteins and etc.)	15-25

the long axis of the tendon (5). The remaining 5-10% of the cellular elements of tendon consists of chondrocytes at the bone attachment and insertion sites (6), synovial cells of the tendon sheath, and vascular cells, including capillary endothelial cells and smooth muscle cells of arterioles (7). Recently, several studies have shown that multipotent tendon stem cells/tendon progenitor cells (TSC/TPC) also exist in human and animal tendon tissues (8-10). Nevertheless, it remains unclear whether the TSC/TPC are the same population of cells as the tenoblast. It is also unclear whether the tenoblast is a committed tenogenic progenitor cell and whether these cells are different from TSC/TPC. At this point there are no known cell markers to differentiate between the tenocyte, tenoblast and TSC/TPC.

In normal tendon, the tenocyte synthesizes a wide range of ECM proteins in a well-ordered structure. Among the most abundant of these proteins is type-I collagen. This protein is organized in a parallel arrangement providing a distinct hierarchical structure, which ultimately forms the tendon (Figure 1). The tenocyte secretes soluble trihelical tropocollagen that is assembled and cross-linked in parallel fibrillar arrays. Higher-order organization of these arrays is provided by the endotenon, which appears as a loose connective tissue layer that envelopes collagen fibrils to form tendon fascicles. Fascicles in turn are bundled together by the epitenon, a layer contiguous with the endotenon through which the microvasculature traverses and provides nutrients (11,12). This multi-unit hierarchical structure aligns fiber bundles parallel with the long axis of the tendon and affords the tendon high tensile strength (1).

Normal tendon ECM is composed largely of collagen (predominantly type-I collagen, COL-I[1]), which provides structural integrity and mechanical strength (13). A small amount of ground substances (Table 1) is not only important in fibrillogenesis but also provides tendon its high resistance behaviour to compressive and tensile forces (14). COL-I constitutes about 60% of the dry mass of the tendon and about 95% of the total collagen in tendon (15). The remaining 5% consists of type III and V collagens. In a normal tendon, type III collagen (COL-III) is mainly located in the endotenon and epitenon (16,17). The ratio of COL-I to COL-III has been previously used as indicators of the tenogenic characteristics in tendon tissues and tenocyte cultures (18,19). Other collagen (types II, VI, IX, X and XI) are present in trace amount in tendons (6). The ground substance of the tendon ECM network surrounding the collagen and tenocytes is composed of proteoglycans and several other small molecules (7). The proteoglycan content in a tendon (dry mass) is relatively lower than other musculoskeletal tissue (14). The content varies at different sites of the tendon and is dependant on the mechanical loading conditions, eg. tension vs. compression (20-22) [~6% in the compression region and ~0.2% in the tensional region]. A summary of the types of proteoglycans present in tendon is presented in Table 2. Although normal mechanical function of tendon depends on the precise alignment of collagen fibrils, it is proteoglycans that regulate collagen fibrillogenesis. This is achieved via the interaction between the positively-charged groups of collagen fibers and the negatively-charged groups of the glycosaminoglycans (GAGs) in a proteoglycan molecule (14). This, indirectly affects a tendon's functionality. Members of the small-leucine-rich proteoglycan (SLRP) family (eg. decorin, biglycan, fibromodulin and lumican) bind to collagen fibrils and actively participate in fibrillogenesis (23). Depletion of biglycan and fibromodulin affects the TSP/TPC differentiation and impairs tendon formation *in vivo* (8). Other proteins, such as adhesive glycoproteins (eg. fibronectin and thrombospondin) are involved in binding the tenocytes to the collagen fibers

[1] Please note that the abbreviation for the gene is given in italics and the abbreviation for the protein expressed by the gene is given in capital letters.

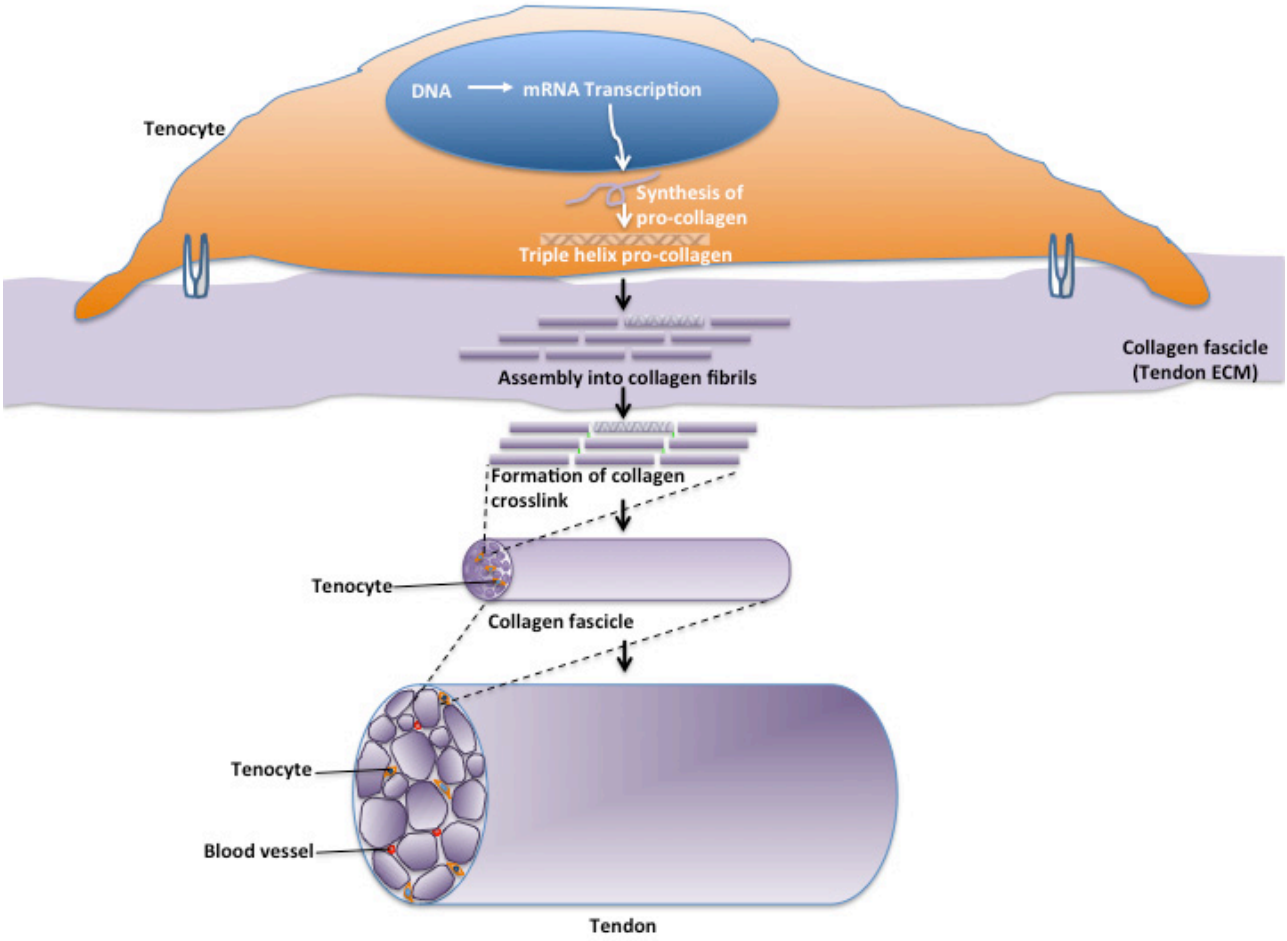

Figure 1: Schematic diagram of hierarchical structure of tendon (68).

The fibril is the smallest tendon structural unit; it consists largely of rod-like collagen molecules aligned end-to-end in a quarter staggered arrays. Fibers form the next level of tendon structure. Fibers are composed of collagen fibrils and are bound by endotenons. Fiber bundles form fascicles, and bundles of fascicles are enclosed by the epitenon. Tendons are also surrounded by a third layer of connective tissue called paratenon (not shown in this figure).

(24). These, are important in the repair and regeneration process in tendon (25-27). Apart from these ECM proteins, several polypeptide factors are important in regulating the expression of specific genes that are commonly found in tendons and the expression of these genes influences the ECM metabolism and subsequently modulates the composition and organization of the tendon ECM (Table 3).

Early Tendon Development

The formation of the musculoskeletal system from the somatic mesoderm requires the coordinated development of muscle, cartilage and tendon lineages. In the early somite development, muscle and cartilage emerge from two distinct compartments, the myotome and the sclerotome. This is in response to signals secreted from the surrounding tissues. As the somite matures, the tendon lineage is established within the dorsolateral sclerotome (or syndetome, the fourth somitic compartment (28)), which is adjacent to and beneath the myotome. The formation of a scleraxis (*Scx*)-expressing tendon progenitor (TP) population in the sclerotome is induced by a fibroblast growth factor (FGF) signal secreted from the myotome.

The FGF transcription effectors (*Pea3* and *Erm*) are necessary for TP marker *Scx* expression in the somite to be expressed (29,30). The domain of *Scx* expression, or the location of the syndetome, is dependent on the combined conditions of the restricted expression pattern of *Pea3* and *Erm* within the anterior and posterior sclerotome, and the distances that FGFs secreted from the center of the myotome are able to travel. Brent and colleagues (2005) also suggested that the early myotome regulatory factors, *Myf5* and *Myod1* (previously known as *MyoD*) expressions are required for FGF protein expression in the myotome, which in turn is required for the induction

Table 2: Summary of most abundant tendon proteoglycans.

Class	Designation	Role in Tendon
SLRP	Decorin	Binds to fibrillar collagen, inhibits collagen fibrillogenesis, binds TGF, and EGF (70).
	Biglycan	Binds to fibrillar collagen, absent in avian species (23).
	Fibromodulin	Binds to type I collagen, facilitates formation of mature large collagen fibrils, modulation of tendon strength (71).
	Lumican	Binds to type I collagen, inhibits size of collagen fibrils, modulation of tendon strength (71).
Modular (lectican)	Aggrecan	Linked to hyaluronan, provides resiliency, low levels in tensional parts of tendon, high levels in compressed regions, particularly in fibrocartilage (72).
	Versican	Linked to hyaluronan, low levels in tensional parts of tendon, somewhat higher levels in compressed regions, increases viscoelasticity, maintains cell shape (73).

Table 3: Genes involved in tendon development and repair (Adapted from James *et al*., 2008) (74).

Gene	Function in development, repair or tissue regeneration
Scleraxis (*Scx*)	Molecular regulator of tenocyte differentiation (75) and activate the *Col-1a1* gene in tendon fibroblast (76).
Tenomodulin (*Tnmd*)	A regulator of cell proliferation, differentiation and collagen fibril maturation (77).
Tenascin C (*Tnc*)	A mechano-responsive modulator of matrix formation expressed in high tensional loading tissue such as tendons and ligaments (78). An ECM protein that is evident during embryonic and tendon development (79).
Collagen I (*Col-I*)	Mature and highly organized collagen fibrils that allows tendon to withstand high tensional loading (76).
Collagen III (*Col-III*)	Early ECM collagen in wound repair (19,80).
Decorin (*Dcn*) and aggrecan (*Acan*)	Proteoglycan interactions modulating collagen fibril orientation and alignment (81).
Smad8	Tenocyte differentiation, phenotype modulation and intracellular signaling (82).

of TP markers. In addition, they suggested that tendon and cartilage lineages arising from the sclerotome appear to be alternative and mutually exclusive, where the loss of chondrocyte differentiation results in an expanded somitic TP population. This causes the *Sox9*-expressing mesenchymal condensations to begin expressing tendon markers. It worth noting that when the differentiation of one cell fate is blocked, the other is adopted (30).

In contrast to the differentiation of axial tendons, that of the cartilages or tendons of the appendicular skeleton arises *in situ*. The initiation of tendon differentiation in the appendicular skeleton does not seem to require the presence of muscle (31). Nevertheless, the maintenance of distal tendons does require interaction with muscle because in the absence of muscle these tendons gradually degenerate (31). Based on the observation of *Scx* expression in the subectodermal location of the appendicular skeleton, it has been postulated that ectodermal signals might play a role in the occurrence of *Scx*-expressing TPs (32). However, the signals that initiate the expression of *Scx* in the appendicular skeleton remain unknown.

In addition to FGF signaling for inducing sclerotomal cells to become tendon progenitor cells (TPC), transforming growth factor - β (TGFβ) signaling is also a potent inducer of *Scx* both in organ culture and in cultured cells (33). This is said to be essential for the maintenance of the early TPC and has been suggested to mediate the recruitment of additional tendon cells by the adjacent muscles and

cartilage condensations. This recruitment is to establish the connections of tendon primordia with these tissues, and it is an essential event for the subsequent differentiation and growth of mature tendons (33). In coordinating the cartilage and tendon differentiation in the developing limb mesenchyme, TGF-interacting factor, *Tgif1*, has been identified as one of the potential candidates which modulates the TGFβ signaling from chondrogenesis to fibrogenesis, and its expression pattern in the limb marks the developing tendons (34). This reprogramming of TGFβ signaling provokes down-regulation of *Sox9* and aggrecan and up-regulation of *Scx* and tenomodulin through the Smad pathway (34). A recent review on the musculoskeletal assembly in the vertebrate embryo postulated that the induction and differentiation of TPCs occur in three distinct stages (Figure 2): induction, organization as well as aggregation and differentiation (35). In brief, the differentiation of tendon in the somite depends upon a combination of both activating and repressing signals from the other compartments of the somite.

However, little is known about other TGF-β family members, in particular the bone morphogenetic protein

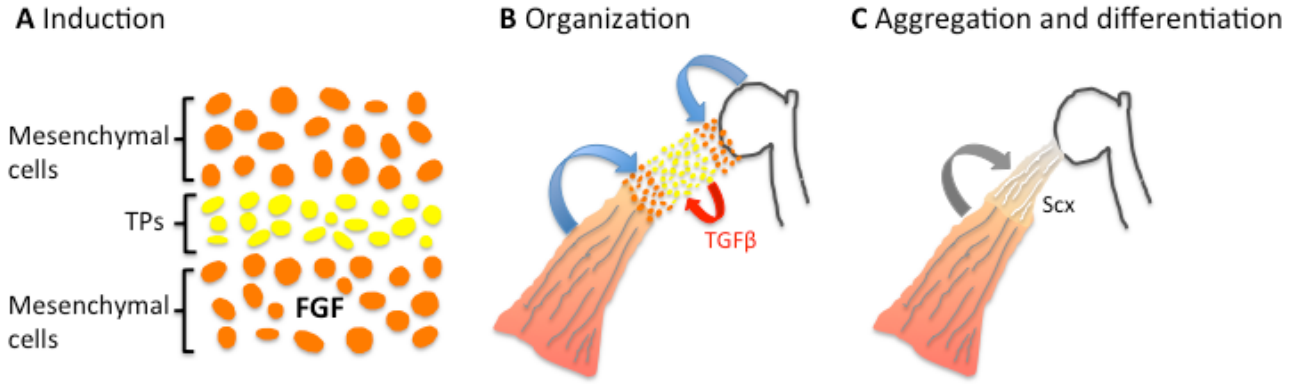

Figure 2: The three main stages and regulators of tendon induction and differentiation in vertebrate embryos (Adapted from Schweitzer *et al*. 2010) (35).

The *Scx*-expressing tendon progenitors (TPs) are represented in yellow and mesenchymal cells in orange to show the different stages of tendon induction and differentiation.

(a) Induction. The initial induction of *Scx*-expressing TPs is associated with FGF signaling, and the myotome in somites is the only identified source to date. In somites and digits, the progenitors are induced at or near their functional position between the myogenic and skeletogenic cells, but in the early limb bud and branchial arches the site of progenitor induction is not related to their final destination.

(b) Organization. At this stage, TPs throughout the embryonic body organize as loose cellular aggregations between the differentiating muscle and skeletal tissues. This transition depends on TGFβ signaling, which mediates the recruitment of additional TPs by the muscle and cartilage tissue to position and integrate the TPs with their interacting musculoskeletal tissues (blue arrows). In addition, TGFβ ligands expressed by the TPs are likely to contribute to the maintenance of the tenoblastic identity of the TPs (red arrow).

(c) Aggregation and differentiation. By E13.5, the TPs condense and organize into structurally distinct tendons that connect to the muscle and cartilage. In some, but not all tendons, tenocyte differentiation depends on *Scx* function. In most tissues, tendon differentiation depends on the presence of muscle (arrow), but the extensor and flexor tendons that extend into the autopod differentiate as structurally distinct tendons even in the absence of muscles.

(BMP) family members in musculoskeletal development. BMP5 is expressed in precise domains in the developing muscle masses and in the autopodial tendons. In the limb mesoderm, Smad and MAPK pathways act synergistically in the BMP pathway controlling limb development (36). Other BMP family members include growth and differentiation factor (GDF) isomers such as GDF5, -6 and -7 (also known as BMP 14, 13 and 12) have also been implicated in tendon development and healing (37-39). Mice deficient GDF5, -6 or -7 exhibit tendon ultrastructural, biological and/or biochemical abnormalities (38,39), whereas exogenous delivery of these factors causes ectopic tendon formation (40). In addition, as one of the earliest known markers of joint formation (37,41), GDF5 dysregulation is strongly linked to various musculoskeletal malformations. GDF5 expression/activity is important in controlling different stages of skeletogenesis, in particular chondrogenesis in a GDF5 dose-dependent manner (42). In cartilage development, GDF5 signaling has a characteristic development pattern in pre-cartilage condensations and in the developing cartilaginous joints (37). Mutations in either GDF5 or its receptor BMP receptor 1B (BMPR1B) lead to similar skeletal malformation phenotypes, indicating that in chondrogenesis, GDF5 signaling seems to be exclusively

mediated through BMPR-1B (43). Many developmental processes, including limb skeletogenesis, also require the segregation of signaling molecules into gradient or the functional compartmentalization of one cell type from another to generate information for differentiation and morphogenesis. Although GDF5 has functional roles in both tendon and cartilage development, it remains unclear whether GDF5 plays a role similar to that of FGF. It may be the case that tendon and cartilage lineages develop in an alternative and mutually exclusive manner through functional compartmentalization processes.

TENDON DAMAGE AND REPAIR MECHANISM

A. Tendon Injury

Tendon injuries, specifically at the shoulder, are a common cause of morbidity and contribute a significant health burden to society. It is defined as a loss of cells or ECM caused by trauma (44). Injury represents a failure of cell and matrix adaptation to a mechanical loading, in excess of the tolerance level, which can be repetitive or prolonged. In these circumstances, there is an inadequate response from the cells or tissues to the mechanical loading applied. In other words, tendon is injured when it is exposed to forces that damage it. Tendon injury at the shoulder can be as the result from forces that cause elongation of the tendon tissue extending into the micro- and macro-failure region. Under physiological circumstances, tendons function in the toe and linear region of the stress-strain curve. Repeated and prolonged load application has been shown to alter the stress-strain curve of the tendon tissue, where tendon injury may result from repeated loading into what would normally be the higher linear region of that curve (1). Rapid unloading has also been associated with tendon injury. Sudden force release is suggested to break interfibrillar adhesion because of shearing force within the tendon (7). In addition to forces that are too big for the tissue to withstand, tendon can also be injured when “normal” forces are applied. This occurrence can be seen in genetic disorders, aging, vascular changes, endocrine influences, nutritional deficiencies, inactivity, immobilization and exercise (45).

The cellular events in ruptured tendon (i.e. rotator cuff tendon) are closely related to the composition and integrity of ECM structure (21,46,47). Tendon ECM transmits mechanical loads, stores and dissipates loading-induced elastic energy. Mechanical deformation in the ECM can transmit forces through tendon cell actin cytoskeleton and cause the remodeling of the actin cytoskeleton (48,49). The cytoskeleton remodeling in turn controls the cell shape, affects cell motility and mediates various cellular functions including DNA and protein synthesis (50). Tendon cells sense mechanical force and convert them into biochemical signals via mechanotransduction mechanisms that ultimately lead to the physiological adaptiveness of tissue or conversely result in pathological changes.

B. Normal Repair Mechanism

Tendon injury will initiate attempts of tissue repair, which has been defined as replacement of damaged or lost cells and ECM by new cells or new matrices (44). In the natural healing process, tendon repair can be divided into different phases (Figure 3). Generally, it consists of an inflammatory phase, proliferation phase, differentiation phase and remodelling phase. In brief, the healing process starts with a hematoma, platelet activation and invasion of cells that form a granuloma. Inflammation after injury protects the body by eliminating and diluting harmful agents, preventing further injury, supplying large quantities of oxygen and nutrients needed for repair, and allowing the entry of clotting agents. Inflammation is triggered by several chemical mediators such as histamines, kinins, prostaglandins, complement, and lymphokines (51).

During the repair process, the clot formed during inflammation is transformed into granulation tissue. The circulating monocytes then differentiate into macrophages after entering the extravascular space. These macrophages are capable of digesting and removing the clot while providing a continuing source of growth factors, chemoattractants, and proteolytic enzymes as needed for tenocyte activation (44). The macrophage-derived growth factor and TGFβ cause the proliferation of tenoblasts originated in the epitenon (52). As tenoblasts infiltrate the wound, blood vessels are formed and facilitate RBC to carry oxygen and nutrients to the developing tissue. Tenoblasts rapidly produce COL-III, which is characterized by smaller fibrils lacking cross-links, which means that the tissue will be lacking tensile strength. At the later stage of this phase, the tenoblasts shift to produce COL-I. Initially, no cross-link occurs between the tropocollagen molecules. This facilitates the enzymatic breakdown and reorganization in the repaired tendon. Cross-links start to develop at 6-14 days post injury increasing tensile strength to the area of injury. At approximately 48 hours to 8 weeks post-injury, the disorganized collagen fibril deposition lies parallel to tensile forces within the tissue.

In the maturation and remodelling phase, cellularity and synthetic activity decreases in the tendon. However, the collagen production has been shown to be 15 times of normal tendon. The granulation tissue is supplanted by new collagen synthesis and deposition, as well as by remodelling myofibroblasts (which derived from the tenoblast that migrated from the edge of wound) that contract the matrix along the axis of the tendon. The ECM becomes more organized at this stage. Wound healing cells and their matrix exist in a dynamic reciprocity whereby cells deposit new matrix and that the matrix modulates gene expression and cell-matrix receptors (53). Through cell-cell and cell-matrix interactions, collagen fibrils align with tenocytes and join end-to-end with other fibrils in the wound and at the margin via covalent crosslinks (2). Most cells (endothelial cells, macrophages and myofibroblasts) then enter apoptosis (programmed cell death), the ECM

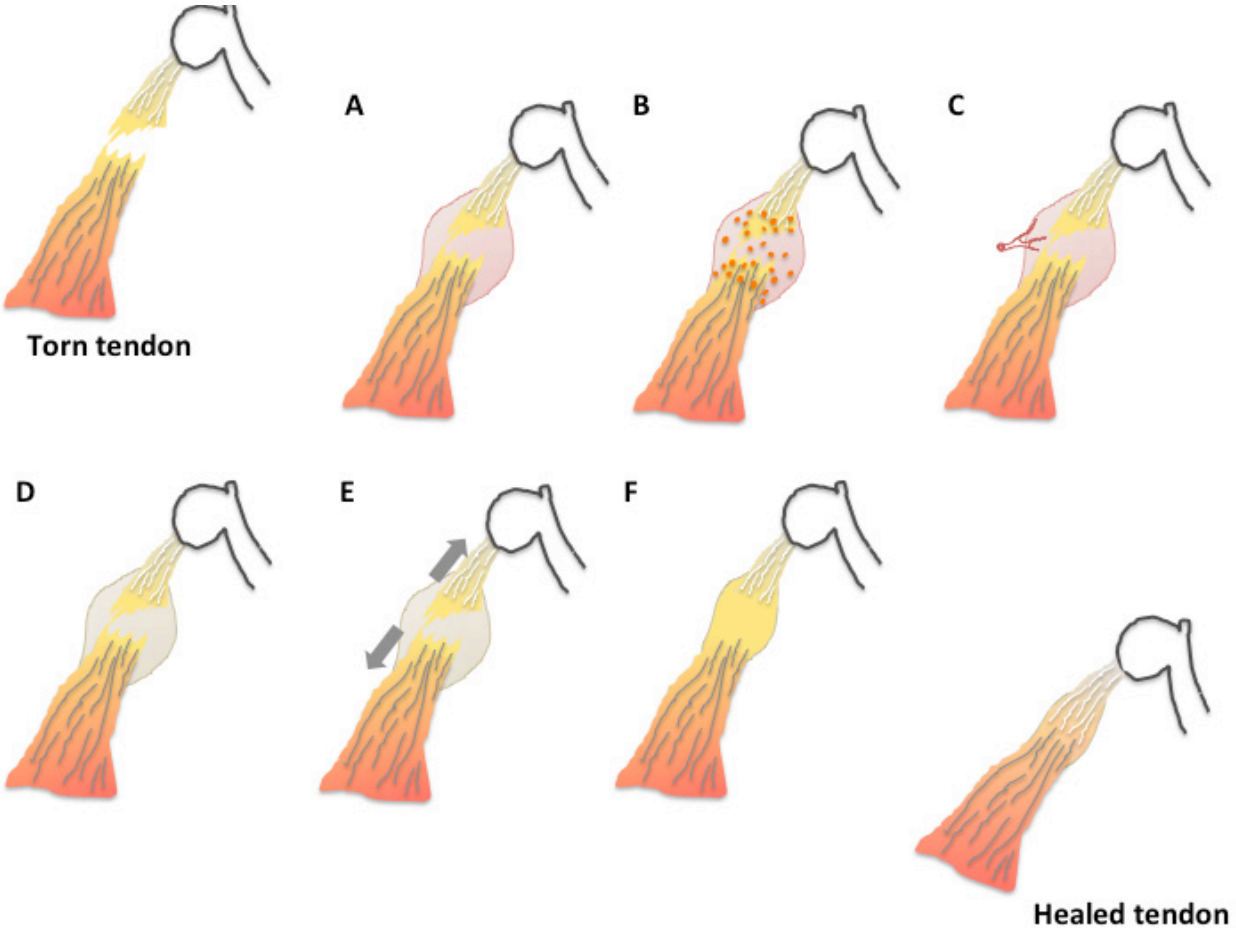

Figure 3: Schematic diagram of tendon repair (69).

(a) Haematoma with platelet activation (inflammatory phase).

(b) Invasion of cells and proliferation of paratenon (proliferation phase).

(c) Vascular and neuronal ingrowth.

(d) Loose collageneous callus formation (differentiation phase).

(e) Mechanical stimulation.

(f) Maturation and remodeling (remodelling phase).

thereby undergoes a transition from a highly cellular granulation tissue to a less densely populated scar tissue (53). Consequently, tendons usually heal with fibrosis and scar tissue, which may regain only 70-80% of their original structural and biomechanical integrity for as long as one-year-post injury. The healed tendon (with suboptimal tensile strength) is prone to reinjury, resulting in lifestyle changes with activity restriction. Poor vascularization (54) and histopathological changes (55) have been suggested as factors contributing to the resulting tendon thickening, fibrosis and being less resistant to tensile stress compared to its preinjured state. The origin of the cells responsible for repairing an injured tendon is controversial. Two mechanisms have been postulated: intrinsic and extrinsic. The former postulates that fibroblast populations come from the endotenon and epitenon, whereas the latter postulates that inflammatory cells and fibroblasts migrate in from surrounding tissues (56). However, a recent report suggested that intrinsic repair may require a progenitor class with predominant tendon marker expression, while extrinsic repair may involve a progenitor class recruited from perivascular cells of the peritenon (57). Tendon TSC/TPC decreases with age and alludes to its association with the age-related reduction in tendon repair as seen in rotator cuff tears (58). Molecular mechanisms controlling these events, either via tenocytes, tenoblast or/and TSC/TPC, and whether a fully differentiated replacement tendon forms at these sites remains largely unclear. The understanding of molecular mechanism in tendon development could assist us in better understanding of tendon etiology and repair.

C. Surgical Repair and Cell Based Therapy in Tendon Healing

Clinically, tendons are repaired or reconstructed using a variety of traditional and innovative methods or surgical techniques that vary with tendon location. These techniques, usually with application of tendon grafts (Table 4), demonstrate various degrees of success. In the light of current shortcomings of tendon repair, the current focus in tissue engineering research is to investigate a repair method which can restore the tissue defects with living cells, or a cell based therapy. A number of cell sources have been suggested, however each cell type demonstrate its own advantages and shortcomings as summarized in Table 5.

Table 4: The advantages and disadvantages of various type of tendon augmentation grafts.

Graft Type	Source	Advantages	Disadvantages
Autograft	Human	No disease transmission risk. No storage required. No preservation problem.	Donor site complication (83,84). Limited availability.
Allograft	Human	No donor site complications. Availability.	Immunogenicity problem (85-87). High risk of disease transmission (87). Required proper storage or preservation (88).
Xenograft	Animal	As with allograft above.	As with allograft above. Ethical issue, i.e. inappropriate animal source such as porcine derived tissue graft.
Prosthesis	Human or animal	As with allograft above.	Low mechanical properties (often result in failure of surgery). Non-specific new tissue induction ability. Induce inflammatory response and rejection (89).
Synthetic	Chemical compounds	Stronger mechanical strength and consistency in quality (89).	Low biocompatibility. Induce inflammatory response and rejection (89).

Table 5: A summary of cell therapy of different cell origins (Adapted from Obaid *et al*. 2010) (90).

Cell Type	Source	Advantages	Disadvantages	Study model (Reference)
Mesenchymal stem cells (MSCs)	Bone marrow-derived	Multilineage potential. Hypoimmunogenecity. Increase rate of tendon healing and maturation. Improve biomechanical and histologic properties of the tendon.	Cannot control differentiation into undesired tissue lineage such as bone, cartilage and muscle. Cell population diminished with age.	Rabbit (91-94) and Mice (94)
	Adipose tissue-derived	Widely available. Simple to obtain. No morbidity to donor site.	As with bone marrow derived MSCs above. Limited application in tendon therapy.	Equine (95,96)
	Synovium-derived	May promote bone-tendon regeneration.	As with bone marrow derived MSCs above.	Nil (97)
	Muscle-derived	As with bone marrow derived MSCs above.	As with bone marrow derived MSCs above. Limited evidence in tendon therapy.	Nil (98)
Fibroblasts	Skin	Great potential in tendon engineering and tendon repair. Widely available. Relatively noninvasive method for cell harvesting. No significant effect to the donor site. Potential source of cells for storage.	Differentiated cells. Uncertainty about behavior in tendon environment. Unsubstantiated repair process. Qualitative repair.	Human (99)
Tendon progenitor/ stem cells	Tendon	Can develop into tendon like tissue.	Morbidity to donor site. No tenocyte markers. No human studies.	Rat (100)

Table 6: A summary of cell therapy outcomes in clinical trials.

Cell Type	Source	Pathology	Patients/Follow-up	Findings
Mesenchymal stem cells (MSCs)	Bone marrow-derived mononuclear cells (64)	1. Complete rotator cuff tears	1. 14 patients (9 women and 5 men, mean age 59.2 years). 2. Mean preoperative UCLA score was 12±3 3. Follow-up at 12 month. 4. A pilot study (cohort study).	1. UCLA score after 12-month follow-up period was 31±3.2). 2. MRI showed tendon integrity in all cases (14/14).
	Bone marrow derived connective tissue progenitor cells (CTPs)(67)	1. Rotator cuff tears	1. 23 patients. 2. Cohort Study.	1. There was no statistical significant difference in: (a) Single Assessment Numeric Evaluation score (CTPs, 86.3 +/- 10.5; control, 83.6 +/- 15.1; p =0.54). (b) Range of motion measures (postoperative external rotation: CTPs, 65.0°±20.4°; control, 62.5 °±17.1°; p =0.67). (c) Postoperative forward elevation (CTPs, 163.0°±30.6°; control, 145.7°±41.4°; p =0.12). (d) Postoperative strength measures between groups (median, 5; range, 4-5 in the CTPs group compared with median, 5; range, 4-5 in the control group; $p > 0.05$).
Fibroblasts	Skin derived tenocyte-like cells (63)	1. Refractory patellar tendinopathy	1. 46 tendinopathy patients (60 patellar tendons). 2. Follow-up at 6 months. 3. Randomized control trial, level of evidence 1.	1. Victorian Institute of Sport Assessment (VISA) score improved from 44±15 before treatment to 75±17 at 6 months post-operative. 2. One patient had a late rupture and progressed to surgery.
	Skin derived tenocyte-like cells (65)	1. Clinical diagnosed refractory lateral epicondylitis.	1. 12 patients (7 women and 5 men). 2. Follow-up at 6 weeks, 3 months and 6 months. 3. Prospective clinical pilot study.	1. The median PRTEE score decreased from 78 before the procedure to 47 at 6 weeks, 35 at 3 months and 12 at 6 months after the procedure (p<0.05). 2. One patient proceeded to surgery after failure of treatment at the end of 3 months.
Tenocyte	Tendon derived tenocyte (66)	1. Rotator cuff tendon injury.	1. A 20-year-old gymnast presented with 12 months of increasing pain during gymnastics being unable to perform most skills. 2. Case study with one year follow-up.	1. At one year after autologous tenocyte implantation, the patient reported substantial improvement of clinical symptoms. 2. Pretreatment and follow-up MRIs scored independently by two musculoskeletal radiologists reported improvement in the tendinopathy and healing on the partial-thickness tear.

Cell based therapy seeks to enhance tissue repair by providing a cell and/or biological scaffold to a repair site in an attempt to elicit a healing response. In order to achieve this, investigators have seeded differentiated cells (mature cells or tenocytes) (59) and undifferentiated cells (mesenchymal stem cells) (60) on scaffolds to develop tissue engineered constructs. Various stimulations, either chemical (using growth factors and cytokines) (61) or mechanical (by stretching) (62), which can mimic the nature of normal tendon *in vivo* environment have been used to enhance the properties of the constructs. Advances in tendon tissue engineering approaches potentially yield a cell-based product that can markedly advance the repair of this soft tissue. Preclinical studies have shown the potential for cellular therapies to increase the tenocyte cell numbers and regenerate rather than repair tendon tissue (Table 5). To date, only 5 clinical studies of cell based therapy in tendons have been reported (Table 6). Of the five human studies reported, only one was randomized control trial, which showed the skin-derived tenocyte-like cells has a better potential than the autologous plasma to improve pain and function in patellar tendinopathy (63). Cohort studies showed that the bone marrow-derived mononuclear cells (64) and skin derived tenocyte-like cells (65) have a potential to improve rotator cuff tear and in lateral epicondylitis respectively. One case study using the ultrasound-guided autologous tenocyte implantation (ATI) showed an improve partial-thickness tear in a gymnast, who was able to return to national-level competition post-ATI (66). Nevertheless, one cohort study reported no significant improvement in the rotator cuff tear treated with bone marrow derived connective tissue progenitor cells (67).

Although current evidence shows that stem cells and tenocytes or tenocyte-like cells can have a positive effect on tendon healing, it remains to be elucidated whether the transplanted cells can help to produce tissue similar to the preinjury state. Questions remain whether tendon development events would re-occur and regenerate tendon tissue, when these cells (stem cells or TP cells) were transplanted to the defect site? In the course of cell-based therapy, would the implanted cells (stem cells, TP cells, tenoblast and tenocytes) together orchestrate cellular events of tendon regeneration? A better understanding in the cellular events involved in tendon development, differentiation and repair is needed in order to lead us to better outcomes for treating tendon injury. The use of adjuncts such as molecular signaling, mechanical stimulation, and other augmentation devices can potentially enhance stem cell therapy in the future.

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