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Review Article

Hylonome

Biology of tendon injury: healing, modeling and remodeling

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Abstract

Tendon disorders are frequent, and are responsible for much morbidity both in sport and the workplace. Although the presence of degenerative changes does not always lead to symptoms, pre-existing degeneration has been implicated as a risk factor for acute tendon rupture. The term **tendinopathy** is a generic descriptor of the clinical conditions in and around tendons arising from overuse. The terms "tendinosis" and "tendinitis/tendonitis" should only be used after histopathological examination. Disordered healing is seen in tendinopathy, and inflammation is not typically seen. In acute injuries, the process of tendon healing is an indivisible process that can be categorized into three overlapping phases for descriptive purposes. Tendon healing can occur intrinsically, via proliferation of epitenon and endotenon tenocytes, or extrinsically, by invasion of cells from the surrounding sheath and synovium. Despite remodeling, the biochemical and mechanical properties of healed tendon tissue never match those of intact tendon. Tendon injuries account for considerable morbidity, and often prove disabling for several months, despite what is considered appropriate management[1]. Chronic problems caused by overuse of tendons probably account for 30% of all running-related injuries[2], and the prevalence of elbow tendinopathy in tennis players can be as high as 40%[3]. The basic cell biology of tendons is still not fully understood, and the management of tendon injury poses a considerable challenge for clinicians. This article describes the structure of tendons, and reviews the pathophysiology of tendon injury and healing.

Keywords: Tendon Injury, Tendinopathy, Rupture, Recovery

Tendon structure

Tendons vary in form, and can be rounded cords, strap-like bands or flattened ribbons[4]. When healthy they appear brilliant white, and have a fibroelastic texture. Structurally, tendon is composed of tenoblasts and tenocytes lying within a network of extracellular matrix (ECM). Tenoblasts are immature tendon cells. They are spindle-shaped, with numerous cytoplasmic organelles reflecting their high metabolic activity[5]. As they age, tenoblasts become elongated and transform into tenocytes[5]. These have a lower nucleus-to-cytoplasm ratio than tenoblasts, with decreased metabolic activity[5]. Together, tenoblasts and tenocytes account for 90-95% of the cellular elements of tendons[5]. The remaining 5-10% of the cellular elements of tendons consists of chondrocytes at the bone attachment and insertion sites, synovial cells of the tendon sheath, and vascular cells, including capillary endothelial cells and smooth muscle cells of arterioles[6].

Tenocytes synthesize collagen and all components of the ECM, and are also active in energy generation[7]. The aerobic Krebs cycle, anaerobic glycolysis and the pentose phosphate shunt are all present in human tenocytes[8]. With increasing age, metabolic pathways shift from aerobic to more anaerobic energy production[9].

Oxygen consumption by tendons and ligaments is 7.5 times lower than skeletal muscles[10]. Given their low metabolic rate and well-developed anaerobic energy generation capacity, tendons are able to carry loads and maintain tension for long periods, whilst avoiding the risk of ischaemia and subsequent necrosis. However, a low metabolic rate results in slow healing after injury[11].

Tenocytes and tenoblasts lie between the collagen fibres along the long axis of the tendon[11]. The dry mass of human tendons is approximately 30% of the total tendon mass, with water accounting for the remaining 70%. Collagen type I accounts for 65-80%, and elastin accounts for approximately 2% of the dry mass of tendons[7].

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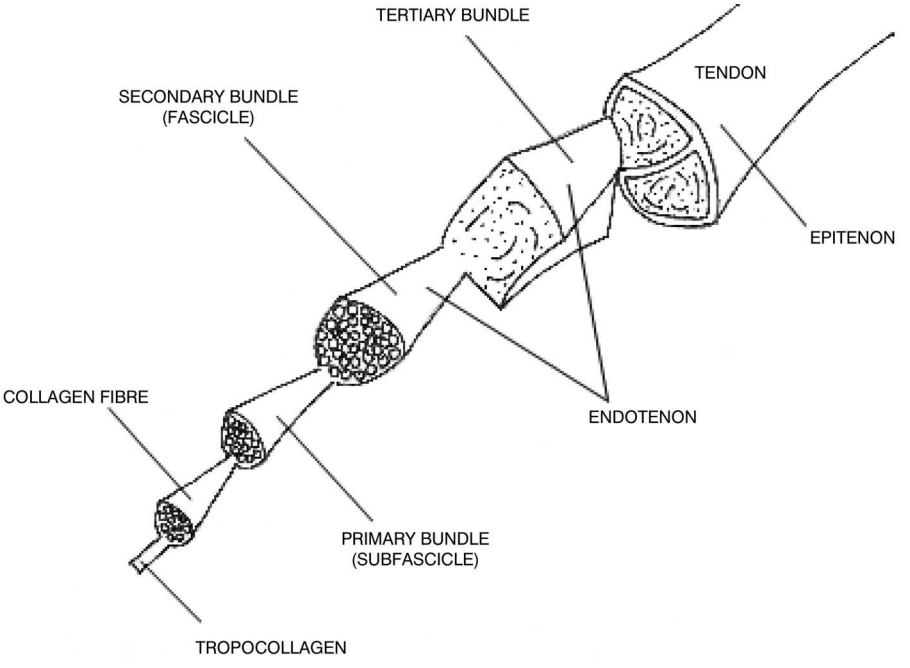

Figure 1. Schematic structure of a normal tendon.

Collagen is arranged in hierarchical levels of increasing complexity, beginning with tropocollagen, a triple-helix polypeptide chain, which unites into fibrils; fibers (primary bundles); fascicles (secondary bundles); tertiary bundles; and the tendon itself (Figure 1)[12]. Soluble tropocollagen molecules form cross-links to create insoluble collagen molecules, which aggregate to form collagen fibrils. A collagen fibre is the smallest tendon unit which can be mechanically tested and is visible on light microscopy. Although collagen fibres are mainly oriented longitudinally, fibres also run transversely and horizontally, forming spirals and plaits[13].

The ground substance of the ECM surrounding the collagen and the tenocytes is composed of proteoglycans, glycosaminoglycans (GAG), glycoproteins and several other small molecules[5]. The strongly hydrophilic nature of proteoglycans enables rapid diffusion of water soluble molecules and migration of cells. Adhesive glycoproteins, such as fibronectin and thrombospondin, participate in repair and regeneration processes in tendon[13]. Tenascin-C, another important component of the tendon ECM, is abundant in the tendon body and at the osteotendinous (OTJ) and myotendinous (MTJ) junctions[14]. Tenascin-C contains a number of repeating fibronectin type III domains, and, following stress-induced unfolding of these domains, it also functions as an elastic protein[14]. The expression of Tenascin-C is regulated by mechanical strain, and is up-regulated in tendinopathy[15]. Tenascin-C may play a role in collagen fibre alignment and orientation[16].

The epitenon, a fine, loose connective-tissue sheath containing the vascular, lymphatic, and nerve supply to the tendon, covers the whole tendon and extends deep within it between the tertiary bundles as the endotenon. The endotenon is a thin reticular network of connective tissue investing each tendon fibre[17]. Superficially, the epitenon is surrounded by paratenon, a loose areolar connective tissue consisting of type I and III collagen fibrils, some elastic fibrils, and an inner lining of synovial cells[8]. Synovial tendon sheaths are found in areas subjected to increased mechanical stress, such as the tendons of the hands and feet, where efficient lubrication is required. Synovial sheaths consist of an outer fibrotic sheath, and an inner synovial sheath, which consists of thin visceral and parietal sheets[12]. The inner synovial sheath invests the tendon body, and functions as an ultrafiltration membrane to produce synovial fluid[18]. The fibrous sheath forms condensations, the pulleys, which function as fulcrums to aid tendon function[19].

At the MTJ, tendinous collagen fibrils are inserted into deep recesses formed by myocyte processes, allowing the tension generated by intracellular contractile proteins of muscle fibres to be transmitted to the collagen fibrils[20]. This complex architecture reduces the tensile stress exerted on the tendon during muscle contraction[20]. However, the MTJ still remains the weakest point of the muscle-tendon unit[20].

The OTJ is composed of four zones: a dense tendon zone, fibrocartilage, mineralized fibrocartilage, and bone[21]. The specialized structure of the OTJ prevents collagen fibre bending, fraying, shearing and failure[22].

Blood supply

Tendons receive their blood supply from three main sources: the intrinsic systems at the MTJ and OTJ, and from

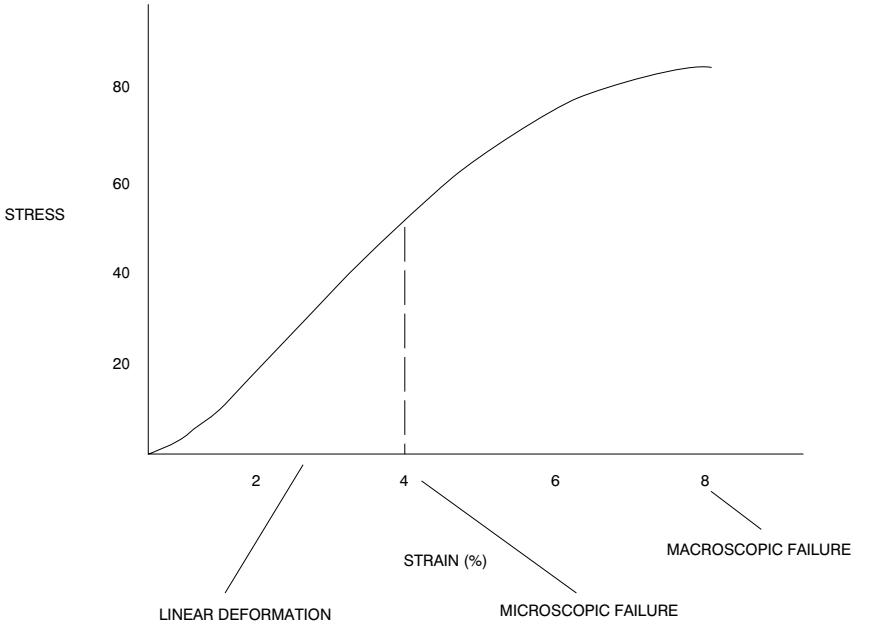

Figure 2. Stress-strain curve demonstrating the basic physical properties of normal tendons.

the extrinsic system via the paratenon or the synovial sheath[23]. The ratio of blood supply from the intrinsic to extrinsic systems varies from tendon to tendon. For example, the central third of the rabbit Achilles tendon receives 35% of its blood supply from the extrinsic system[24]. At the MTJ, perimyseal vessels from the muscle continue between the fascicles of the tendon[23]. However, blood vessels originating from the muscle are unlikely to extend beyond the proximal third of the tendon[23]. The blood supply from the OTJ is sparse, and limited to the insertion zone of the tendon, although vessels from the extrinsic system communicate with periosteal vessels at the OTJ[5,23].

In tendons enveloped by sheaths to reduce friction, branches from major vessels pass through the vincula (mesotenon) to reach the visceral sheet of the synovial sheath, where they form a plexus[12]. This plexus supplies the superficial part of the tendon, while some vessels from the vinculae penetrate the epitenon. These penetrating vessels course in the endotenon septae, and form a connection between the peri- and intra-tendinous vascular networks.

In the absence of a synovial sheath, the paratenon provides the extrinsic component of the vasculature. Vessels entering the paratenon course transversely, and branch repeatedly to form a complex vascular network[25]. Arterial branches from the paratenon penetrate the epitenon to course in the endotenon septae, where an intratendinous vascular network with abundant anastomoses is formed[5,26].

Tendon vascularity is compromised at junctional zones and sites of torsion, friction or compression. In the Achilles tendon, angiographic injection techniques have demonstrated a zone of hypovascularity 2-7 cm proximal to the tendon insertion[23]. However, laser Doppler flowmetry has demonstrated substantially reduced blood flow near the Achilles tendon insertion, with an otherwise even blood flow throughout the tendon[27]. A similar zone of hypovascularity is present on the dorsal surface of the flexor digitorum profundus tendon subjacent to the volar plate, within 1 cm of the tendon insertion[28]. In general, tendon blood flow declines with increasing age and mechanical loading[27], and peak exercise peritendinous blood flow reaches only approximately 20% of the maximal blood flow capacity in that area[29].

Tendon innervation

Tendon innervation originates from cutaneous, muscular, and peritendinous nerve trunks. At the MTJ, nerve fibres cross and enter the endotenon septa. Nerve fibres form rich plexuses in the paratenon, and branches penetrate the epitenon. Most nerve fibres do not actually enter the main body of the tendon, but terminate as nerve endings on its surface.

Nerve endings of myelinated fibres function as specialised mechanoreceptors to detect changes in pressure or tension. These mechanoreceptors, the Golgi tendon organs, are most numerous at the insertion of tendons into the muscle[30]. Golgi tendon organs are essentially a thin delicate capsule of connective tissue that enclose a group of branches of large myelinated nerve fibres. These fibres terminate with a spray of fibre endings between bundles of collagen fibres of the tendon[31].

Unmyelinated nerve endings act as nociceptors, and sense and transmit pain. Both sympathetic and para-sympathetic fibres are present in tendon[32].

Biomechanics

Tendons transmit force generated by muscle to bone, and act as a buffer by absorbing external forces to limit muscle damage[33]. Tendons exhibit high mechanical strength, good flexibility, and an optimal level of elasticity to perform their unique role[34]. Tendons are viscoelastic tissues, which display stress relaxation and creep[35].

The mechanical behaviour of collagen is dependent on the number and types of intra- and inter-molecular bonds[36]. A stress-strain curve helps to demonstrate the behaviour of the tendon (Figure 2). At rest, collagen fibres and fibrils display a crimped configuration[37]. The initial concave portion of the curve (toe region), where the tendon is strained up to 2%, represents flattening of the crimp pattern[38]. Beyond this point, the tendon deforms in a linear fashion due to intramolecular sliding of collagen triple helices, and the fibres become more parallel[39]. If the strain remains below 4%, the tendon behaves in an elastic fashion, and returns to its original length when unloaded[40]. Microscopic failure occurs when the strain exceeds 4%, and, beyond 8-10% strain, macroscopic failure occurs from intrafibril damage by molecular slippage[41]. X-ray diffraction studies have demonstrated that collagen fibril elongation initially occurs due to molecular elongation, but, as stress increases, the gap between molecules increases, eventually leading to slippage of lateral adjoining molecules[41]. After this, complete failure occurs rapidly, and the fibres recoil into a tangled bud at the ruptured end[33].

The tensile strength of tendons is related to thickness and collagen content, and a tendon with an area of 1 cm^2 is capable of bearing 500-1,000 kg[42]. During strenuous activities such as jumping and weight lifting, very high loads are placed on tendons[43]. In the human Achilles tendon, forces of 9 kN, corresponding to 12.5 times body weight, have been recorded during running[44]. Since these forces exceed the single-load ultimate tensile strength of the tendon, the rate of loading may also play an important role in tendon rupture[38]. Using non-invasive means, the mechanical properties of superficial tendons based on stress-strain curves can now been performed in humans *in vivo*[45].

Tendons are at the highest risk for rupture if tension is applied quickly and obliquely, and highest forces are seen during eccentric muscle contraction[36].

Physiological responses of tendon

In animal experiments, training results in improved tensile strength, elastic stiffness, weight and cross-sectional area of tendons[46]. These effects can be explained by an increase in collagen and ECM synthesis by tenocytes[46]. Little data exist on the effect of exercise on human tendons, although intensively trained athletes are reported to have thicker Achilles tendons than control subjects[47,48]. Most of the current knowledge is therefore based on the result of animal studies[49]. However, care must be taken when interpreting animal studies, as untrained animals may be compared to trained animals. Also, confined animals are likely to have reduced connective tissue mass and tensile strength, and physical training may merely return this to normal[38].

Prolonged immobilization following musculoskeletal injury often results in detrimental effects. Collagen fascicles from stress shielded rabbit patellar tendons display lower tensile strength and strain at failure than control samples[50]. Immobilization reduces the water and proteoglycan content of tendons, and increases the number of reducible collagen cross-links[51]. Immobilization results in tendon atrophy (Maganaris et al., 2005), but, due to low metabolic rate and vascularity, these changes occur slowly[48,52].

Tendon properties and function also deteriorate with ageing. Muscle strength and power decline[53]. This is thought to be due to a loss of collagen and its cross-linking resulting in an increase in tendon stiffness[54]. Resistance training in old age can partly reverse the deteriorating effect of ageing on tendon properties and function[55,56].

Tendon injury

Tendon injuries can be acute or chronic, and are caused by intrinsic or extrinsic factors, either alone or in combination. In acute trauma, extrinsic factors predominate, whilst in chronic cases intrinsic factors also play a role.

Tendinopathy

In chronic tendon disorders, interaction between intrinsic and extrinsic factors is common[11]. Intrinsic factors such as alignment and biomechanical faults are claimed to play a causative role in two-thirds of athletes with Achilles tendon disorders[57]. In particular, hyperpronation of the foot has been linked with an increased incidence of Achilles tendinopathy[58].

Excessive loading of tendons during vigorous physical training is regarded as the main pathological stimulus for degeneration[59]. In the presence of intrinsic risk factors, excessive loading may carry a greater risk of inducing tendinopathy. Tendons respond to repetitive overload beyond physiological threshold by either inflammation of their sheath, degeneration of their body, or a combination of both[60]. Different stresses induce different responses. Active repair of fatigue damage must occur, or tendons would weaken and eventually rupture[61]. The repair mechanism is probably mediated by resident tenocytes, which maintain a fine balance between ECM production and degradation. Tendon damage may even occur from stresses within physiological limits, as frequent cumulative microtrauma may not allow enough time for repair[62]. Microtrauma can also result from non-uniform stress within tendons, producing abnormal load concentrations and frictional forces between the fibrils, resulting in localised fibre damage[62].

The aetiology of tendinopathy remains unclear, and many causes have been theorised. Hypoxia, ischaemic damage, oxidative stress, hyperthermia, impaired apoptosis, inflam-

matory mediators, fluoroquinolones, and matrix metalloproteinase imbalance have all been implicated as mechanisms of tendon degeneration[6,63-70].

Histologically, tendinopathy shows a picture of disordered haphazard healing with absence of inflammatory cells, poor healing response, non-inflammatory intratendinous collagen degeneration, fibre disorientation and thinning, hypercellularity, scattered vascular ingrowth, and increased interfibrillar glycosaminoglycans[12]. Frank inflammatory lesions and granulation tissue are infrequent, and are mostly associated with tendon ruptures[71].

Macroscopically, the affected portions of the tendon lose their normal glistening white appearance and become grey-brown and amorphous. Tendon thickening, which can be diffuse, fusiform or nodular, occurs[72]. Tendinosis is often clinically silent, and its only manifestation may be a rupture, but it may also co-exist with symptomatic paratendinopathy[64].

Tendon rupture

Tendon rupture is an acute injury in which extrinsic factors predominate, although intrinsic factors are also important. In Achilles tendon rupture, an acceleration/deceleration mechanism has been reported in up to 90% of sports-related injuries[73]. Malfunction of the normal protective inhibitory pathway of the musculo-tendinous unit may result in injury[74]. The aetiology of tendon rupture remains unclear[10]. Degenerative tendinopathy is the most common histological finding in spontaneous tendon ruptures. Arner et al. first reported degenerative changes in all their 74 patients with Achilles tendon rupture, and hypothesised that these changes were due to intrinsic abnormalities present before the rupture[75]. Kannus and Jozsa found degenerative changes in 865 of 891 (97%) spontaneous tendon ruptures, whilst degenerative changes were only seen in 149 of 445 (34%) of control tendons[9]. Tendon degeneration may lead to reduced tensile strength and a predisposition to rupture. Indeed, ruptured Achilles tendons have a histological picture of greater degeneration than chronic painful tendons from overuse injuries[76].

Pain in tendinopathy

Classically, pain in tendinopathy has been attributed to inflammation. However, chronically painful Achilles and patellar tendons show no evidence of inflammation, and many tendons with intratendinous pathology detected on MRI or ultrasound are not painful[72]. Pain may originate from a combination of mechanical and biochemical causes[72]. Tendon degeneration with mechanical breakdown of collagen could theoretically explain the pain, but clinical and surgical observations challenge this view[72]. Chemical irritants and neurotransmitters may generate pain in tendinopathy. Microdialysis sampling revealed a two-fold increase in lactate levels in tendinopathic tendons compared to controls[77]. Patients with chronic Achilles tendinopathy and patellar tendinopathy show high concentrations of the neurotransmitter glutamate, with no statistically significant elevation of the pro-inflammatory prostaglandin PG E_2[78]. However, the levels of PG E_2 were consistently higher in tendinopathic tendons compared to controls, and the results possibly lacked statistical significance due to the small sample size of the study.

Substance P functions as a neurotransmitter and neuromodulator, and is found in small unmyelinated sensory nerve fibres[79]. A network of sensory innervation is present in tendons[80]. Sensory nerves transmit nociceptive information to the spinal cord, and increased levels of substance P correlate with pain levels in rotator cuff disease and medial and lateral epicondylopathy[81,82].

An opioid system exists in the Achilles tendon of rats[83]. Under normal conditions, a balance probably exists between nociceptive and anti-nociceptive peptides[84]. However, this balance may be altered in pathological conditions[84].

Tendon healing following acute injuries

Tendon healing studies have predominantly been performed on transected animal tendons or ruptured human tendons, and their relevance to human tendinopathy with its associated healing failure response remains unclear[85].

Tendon healing occurs in three overlapping phases. In the initial inflammatory phase, erythrocytes and inflammatory cells, particularly neutrophils, enter the site of injury. In the first 24 hours, monocytes and macrophages predominate, and phagocytosis of necrotic materials occurs. Vasoactive and chemotactic factors are released with increased vascular permeability, initiation of angiogenesis, stimulation of tenocyte proliferation, and recruitment of more inflammatory cells[86]. Tenocytes gradually migrate to the wound, and type III collagen synthesis is initiated[87].

After a few days, the remodeling stage begins. Synthesis of type III collagen peaks during this stage, which lasts for a few weeks. Water content and glycosaminoglycan concentrations remain high during this stage[87].

After approximately 6 weeks, the modeling stage commences. During this stage, the healing tissue is resized and reshaped. A corresponding decrease in cellularity, collagen and glycosaminoglycan synthesis occurs. The modeling phase can be divided into a consolidation and maturation stage[88]. The consolidation stage commences at about 6 weeks and continues up to 10 weeks. In this period, the repair tissue changes from cellular to fibrous. Tenocyte metabolism remains high during this period, and tenocytes and collagen fibres become aligned in the direction of stress[89]. A higher proportion of type I collagen is synthesized during this stage[90]. After 10 weeks, the maturation stage occurs, with gradual change of fibrous tissue to scar-like tendon tissue over the course of one year[89]. During the latter half of this stage, tenocyte metabolism and tendon vascularity decline[91].

Tendon healing can occur intrinsically, via proliferation of epitenon and endotenon tenocytes, or extrinsically, by invasion of cells from the surrounding sheath and synovium[92]. Epitenon tenoblasts initiate the repair process through proliferation and migration[93]. Healing in severed tendons can be performed by

cells from the epitenon alone, without relying on adhesions for vascularity or cellular support[94]. Internal tenocytes contribute to the intrinsic repair process and secrete larger and more mature collagen than epitenon cells[95]. Despite this, fibroblasts in the epitenon and tenocytes synthesize collagen during repair, and different cells probably produce different collagen types at different time points. Initially, collagen is produced by epitenon cells, with endotenon cells later synthesizing collagen[96]. The relative contribution of each cell type may be influenced by the type of trauma sustained, anatomical position, presence of a synovial sheath, and the amount of stress induced by motion after repair has taken place[97].

Tenocyte function may vary depending on the region of origin. Cells from the tendon sheath produce less collagen and GAG compared to epitenon and endotenon cells. However, fibroblasts from the flexor tendon sheath proliferate more rapidly[98]. The variation in phenotypic expression of tenocytes has not been extensively investigated, and this information may prove useful for optimizing repair strategies.

Intrinsic healing results in improved biomechanics and fewer complications. In particular, a normal gliding mechanism within the tendon sheath is preserved[99]. In extrinsic healing, scar tissue results in adhesion formation which disrupts tendon gliding[100]. Different healing patterns may predominate in particular locations, and, for example, extrinsic healing tends to prevail in torn rotator cuffs[101].

Remodeling responses

The histopathological process as the basis of the clinical manifestations of tendinopathy then can be viewed as a failure of cell matrix adaptation to a variety of stresses, due to an imbalance between matrix degeneration and synthesis[102]. Remodeling plays an important role in responding to microtrauma from repetitive loading. This repair mechanism is probably mediated by resident tenocytes, which maintain a fine balance between ECM production and degradation.

Modeling is also involved in the physiological response of tendon to resistance training. In such situations, modelling adapts the tendon to the mechanical loads placed on it, and prevents the tendons from incurring injuries. An increase in the tendon mass and cross-sectional area occurs during modeling.

Modulators of healing

MMPs are important regulators of ECM remodeling, and their levels are altered during tendon healing[70]. In a rat flexor tendon laceration model, the expression of MMP-9 and MMP-13 (Collagenase-3) peaked between days 7 and 14. MMP-2, MMP-3, and MMP-14 (MT1-MMP) levels increased after surgery, and remained high until day 28[103]. These findings suggest that MMP-9 and MMP-13 participate only in collagen degradation, whereas MMP-2, MMP-3 and MMP-14 participate in both collagen degradation and collagen remodeling. Wounding and inflammation also provoke the release of growth factors and cytokines from platelets, polymorphonuclear leukocytes, macrophages and other inflammatory cells[104]. These growth factors induce neovascularization and chemotaxis of fibroblasts and tenocytes and stimulate fibroblast and tenocytes proliferation and synthesis of collagen[105].

Nitric oxide is a short-lived free radical, with many biological functions: it is bactericidal, can induce apoptosis in inflammatory cells, and causes angiogenesis and vasodilatation[106,107]. Nitric oxide may play a role in several aspects of tendon healing. Nitric oxide synthase is responsible for synthesizing nitric oxide from L-arginine. Experimental studies have shown that levels of nitric oxide synthase peak after 7 days and return to baseline 14 days after tenotomy of rat Achilles tendons[108]. Inhibition of nitric oxide synthase reduced healing and resulted in decreased cross-sectional area and a reduced failure load[108]. In that study, the specific isoforms of nitric oxide synthase were not identified. More recently, the same group has demonstrated a temporal expression of the three isoforms of nitric oxide synthase[109]. The inducible isoform peaks at day 4, the endothelial isoform peaks at day 7, and the neuronal isoform peaks at day 21[109].

Interestingly, in a rat Achilles tendon rupture model, peak nerve fibre formation occurred between weeks 2 and 6, in concert with peak levels of the neuronal isoform of nitric oxide synthase[110]. These nerve fibres presumably deliver neuropeptides, which act as chemical messengers and regulators, and may play an important role in tendon healing. Substance P and calcitonin gene-related peptide (CGRP) are pro-inflammatory and cause vasodilation and protein extravasation[111,112]. In addition, Substance P enhances cellular release of prostaglandins, histamines and cytokines[113]. Peak levels of substance P and CGRP occur during the proliferative phase, suggesting a possible role during this phase.

Limitations of healing in acute tendon injuries

Adhesion formation after intrasynovial tendon injury poses a major clinical problem[114]. Synovial sheath disruption at the time of injury or surgery allows granulation tissue and tenocytes from surrounding tissue to invade the repair site. Exogenous cells predominate over endogenous tenocytes, allowing the surrounding tissues to attach to the repair site resulting in adhesion formation.

Despite remodeling, the biochemical and mechanical properties of healed tendon tissue never match those of intact tendon. In spontaneously healed transected sheep Achilles tendons, rupture force was only 56.7% of normal at 12 months[115]. One possible reason for this may be the absence of mechanical loading during the period of immobilization.

Conclusion

Tendon injuries give rise to substantial morbidity, and current understanding of the mechanisms involved in tendon injury and repair is limited. Further research is required to improve our knowledge of tendon healing. This will enable specific treatment strategies to be developed[116].

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