Mechanobiological Analyses of Healing Tendons using Computational Approaches

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Abstract

The healing process of ruptured tendons is problematic due to scar tissue formation and deteriorated material properties. In some cases, it may take nearly a year to complete. Mechanical loading has been shown to positively influence tendon healing; however, the mechanisms remain unclear. Computational mechanobiology methods employed extensively to model bone healing have achieved high fidelity, but not yet been explored to understand tendon regeneration. The general objective of this thesis is to develop computational approaches to enhance the knowledge of the role that mechanical factors play in fibre re-organisation in healing tendons, by proposing an appropriate constitutive formulation, followed by analysing the mechano-adaptation of the models created when regulated by different biophysical stimuli. Curve fitting of an established hyperelastic fibre-reinforced continuum model introduced by Gasser, Ogden and Holzapfel (GOH) against experimental tensile testing data of rat Achilles tendons at four timepoints during the tendon repair was used and achieved excellent fits ($0.9903 < R^2 < 0.9986$). A parametric sensitivity study using a three-level central composite design, which is a fractional factorial design method, showed that the collagen-fibre-related parameters in the GOH model had almost equal influence on the fitting. The mechano-adaptation of the healing tendons when regulated by axial and principal strain predicted fibre re-organisation comparable to experimental findings, in contrast to models regulated by deviatoric strain. Also, mechano-adaptive models regulated by deviatoric strain were more spatially and temporally sensitive to different boundary conditions - length and loading magnitudes - than those regulated by axial and principal strain. This thesis describes that a hyperelastic fibre-reinforced mechano-adaptive model regulated by axial or principal strain is generally capable of describing the mechanobiological behaviours of healing tendons, and that further experiments should focus on establishing the localised structural and material parameters of collagen fibres and their mechano-adaptive behaviours in the healing tissue.

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1 Introduction

This chapter includes a short introduction to the problems concerning tendon tissue regeneration, particularly with regard to healing of ruptured tendons. The role of the mechanical environment, both globally and locally, is then introduced with a focus on how computational models can be of assistance. The overall hypothesis and the specific goals of the thesis are described, including the specific research questions investigated in each of the conducted studies. The general methodology is briefly outlined.

1.1 Problem statement

Tendon as a complex tissue - Tendon is a structurally multi-hierarchical tissue with a predominantly mechanical function translating muscular contraction into joint movement by transmitting forces from muscle to bone. It also functions as a buffer to prevent stress concentration at both muscle and bone, by having an intermediate stiffness between that of muscle and that of bone (Wang 2006). Owing to the critical role of tendon in body mechanics, injury and degeneration of tendon can be highly disabling, subsequently resulting in substantial pain and disability (Killian, Cavinatto et al. 2012).

Tendon rupture, the epidemiology - In humans, tendon disease and injury are common and costly, affecting both professional athletes (UK Achilles tendinopathy estimated incidence 500 per year (Jarvinen, Kannus et al. 2005)) and the general population (UK Achilles rupture incidence up to 20 / 100,000 per year (Lantto, Heikkinen et al. 2015)), with earnings loss and painful and prolonged rehabilitation. Recent reports have shown that the number of ruptured tendons has increased over the past decades (Wertz, Galli et al. 2013), attributed to increased sports participation in the occasional athlete (Leppilahti, Puranen et al. 1996; Maffulli, Waterston et al. 1999). Also a retrospective study of the National Hospital Discharge Register in Finland found that urban areas had a higher incidence of ruptures than rural areas (Nyyssonen, Luthje et al. 2008). Given the fact that urban areas, especially those in China, India, and Africa, are gradually expanding while the population is generally becoming older (Seto, Fragkias et al. 2011), the likelihood of having more cases of ruptured tendon in the future is high.

Positive effects of mechanical loading - It is well recognised that mechanical stimulation can affect rupture repair and alter its mechanobiological pathways, and a few studies have shown its positive effects on repair, i.e. resulting in faster recovery and stronger tendons, by preventing adhesions and improving cell activity as well as collagen

deposition (Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Andersson, Eliasson et al. 2012; Killian, Cavinatto et al. 2012; Schepull and Aspenberg 2013; Nourissat, Berenbaum et al. 2015). However, despite extensive studies in this field, the detailed mechanisms by which mechanical stimuli are transferred, via cellular mediators into a tissue response that results in a biochemical response remain unknown.

Mechanobiology and computer simulation - Mechanobiology describes the mechanisms by which biological processes are regulated by signals to cells that are induced by mechanical loads over time (van der Meulen and Huiskes 2002). When the mechanisms of mechanically-regulated tissue formation are understood and well defined at all levels, i.e. organ, tissue and cellular levels, specific treatments may be developed and used to accelerate healing and to prevent scar formation and eventually restore optimal function of the tendon. Many biological processes, including tendon healing, are so complex that physical experimentation is either too time consuming, too expensive, or impossible. Mathematical models, implemented computationally, can simulate these complex systems wherein also allow for systematic parametric analyses defining factors involved in the processes. In mechanobiology, computational models have been developed and used together with in vivo and in vitro experiments, to quantitatively explore and explain the rules that govern the effects of mechanical loading on cells and tissue differentiation, growth, adaptation and maintenance. For example in bone, a mechanoregulatory algorithm regulated by octahedral shear strain and fluid velocity (Prendergast, Huiskes et al. 1997), included in a finite element (FE) model successfully predicted bone regeneration patterns both spatially and temporally, dependent on distraction rate and frequency during distraction osteogenesis (Isaksson, Comas et al. 2007). Another recent example, an arterial tissue model, was developed whereby responses of the vascular cells to both chemical and mechanical environments during growth and

remodelling were included (Aparício, Thompson et al. 2016). The model created successfully predicted different modes of aneurysm development. However, up until now few models have been applied to tendon healing.

The general approach in computational mechanobiology is that mechanical perturbations are applied to model geometry, and the local mechanical environment is calculated, using the FE method. The biological aspects of the computations are based on different premises that local mechanical stimuli induce certain tissue or cellular activities, for example cell proliferation, or changes in collagen fibre orientations. Consequently, this affects the mechanical response at the next loading step. Computational models are gradually becoming more sophisticated with increasing computational power and biological knowledge. Both experimental and computational studies are critical to advance our knowledge in mechanobiology. Integration of these fields is important, since models can help interpret experiments and experiments can provide relationships and observations for model development.

Current problems – In order to investigate the mechanobiological behaviours of healing tendons using computational approaches, a constitutive model that includes quantifiable micro-structural parameters that is also able to capture the biomechanical response is required. This constitutive model should also allow for estimates of potential biophysical stimuli in the healing tendons. As addressed in previous computational works on mechanoregulation of the musculoskeletal system (Hayward and Morgan 2009; Isaksson 2012), the most appropriate tissue level scalar mechanical quantity needs to be identified to provide input into any mechanoregulation algorithm representing the healing process. These forms of investigations have, so far, not been carried out in tendon regeneration.

Importance of the study – An understanding of the principles of tendon repair may have implications beyond treatment of ruptured tendons, with applications in tissue regeneration in general, such as during integration of a tendon graft with its surrounding tissues, i.e. ligament or bone, and in tissue engineering. Tendon also provides a suite of easily accessible in vitro and in vivo models, and can be used to represent general connective tissue containing fibrillar collagen, elastin, and glycosaminoglycans. Additionally, the outcomes from studies on this relatively simple structure may help investigations on more complex and less physically accessible tissues. Therefore, a better understanding of all the factors that influence the tendon healing process in general and its mechanobiology, in particular, will have important applications in musculoskeletal generation and regeneration, and their respective treatment.

1.2 Aims and outline of the thesis

The need to further examine the mechanoregulatory mechanisms of tendon healing has been identified in the previous section. The general objective of this thesis is to develop computational approaches to enhance the knowledge of the role that mechanical factors play in fibre re-organisation in healing tendons, by proposing an appropriate constitutive formulation, followed by analysing the mechano-adaptation of the models created when regulated by different biophysical stimuli. The fundamental hypothesis is that the local level of mechanical stimulation, using strain invariants, determines the collagen fibre re-organisation pathways. The general objectives are divided into specific aims and hypotheses, which are addressed in subsequent chapters. The specific objective as well as the general approach for each chapter is specified below:

Chapter 2 – Mechanobiological behaviour of healing tendons

- To provide a comprehensive literature basis to describe the current knowledge and previous research conducted in the area of biomechanics and mechanobiology of healing tendons.

Chapter 3 – Mechanobiological modelling of tendons: review and future opportunities

- Extensive literature review on existing computational models of biological soft tissue is essential to discover potential models for simulating healing tendons. The aim of this chapter is to provide an overview of the latest developments in computational modelling that can be applied to understanding and harnessing tendon biomechanics and mechanobiology, and lays the foundation for the work in the current thesis.

Chapter 4 – A hyperelastic fibre-reinforced continuum model of healing tendons with distributed collagen fibre orientations

- One well-established formulation based on a fibre-reinforced continuum model with distributed collagen fibres, proposed by Gasser-Ogden-Holzapfel (GOH) (Gasser, Ogden et al. 2006), which has been used to simulate most of soft tissues other than healing tendons, was found plausible. This chapter thus aimed to test the ability of the GOH model in capturing biomechanical behaviours of healing tendons by fitting it with experimental tensile data of rat Achilles tendons, at different timepoints of healing. Experimental data on in vivo Achilles tendon rupture healing was kindly provided by Dr Pernilla Eliasson (2011). The GOH model for healing tendon simulation was further tested in an axisymmetric FE

environment. The sensitivity of the model parameters were also analysed using a design of experiments method.

Chapter 5 – Mechano-adaptive models of healing tendons

- The previous constitutive model was used to develop axisymmetric finite element (FE) mechano-adaptive models, focussing on tissue type turnover based on collagen fibre organisations. This chapter is specifically aimed to propose a computational platform for tendon healing implementing a model for changing fibre dispersion to represent tissue reorganisation, to test its sensitivity to geometric and loading parameters and to evaluate three possible biophysical stimuli's ability to predict the temporal and spatial healing. Following Perren (S.M. Perren and J. Cordey 1980), a mechanoregulatory scheme was adopted that proposed tissue types with higher stiffness could only form at lower strain. This scheme is also suggested by a more recent work showing that tenogenic differentiation requires a stiffer matrix/substrate than granulation tissue (Sharma and Snedeker 2010). Results from in vivo animal experiments were employed for a range of qualitative and quantitative comparative analyses between computational predictions and experimental data.

Chapter 6 – Discussion and conclusions

- To conclude the outcomes and discuss the logic of the thesis as a whole and to incorporate it with past research and future prospects.

The overall approach used in this study is shown in Figure 1.1.

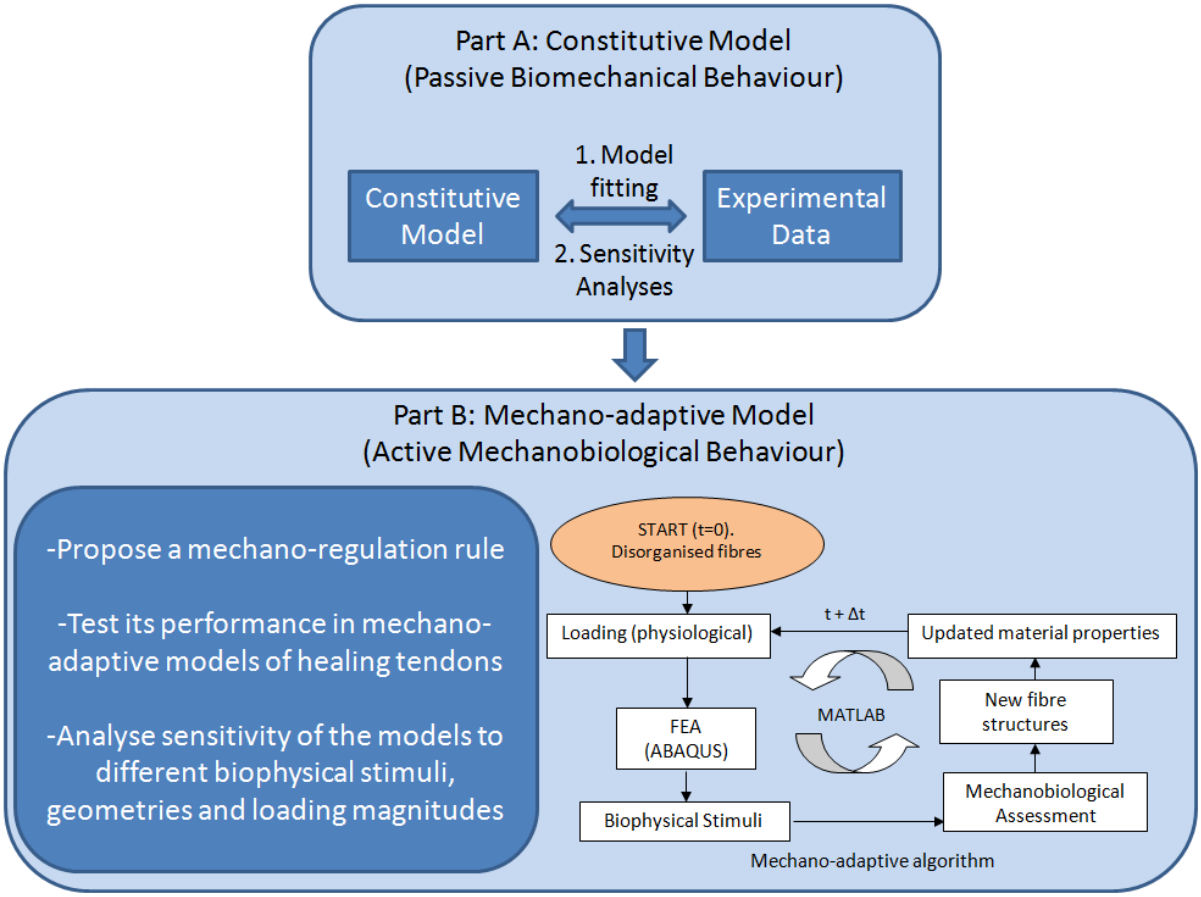


Figure 1.1. General scheme of the approach used in this study.

2 Mechanobiological behaviour of healing tendons

This chapter provides a literature review of the topics addressed in this thesis. It includes a brief description of tendon structure and composition, the biomechanical behaviours and overview of tendon rupture, followed by a description of the mechanisms of healing tendons and their mechanobiological behaviours. Thereafter, mechanotransduction involved in the process of healing and biological treatments for ruptured tendons is summarised.

2.1 Introduction

Tendons are specialised connective tissues that transmit loads from muscle to bone, making them important during locomotion while simultaneously providing joint stability. They are used for energy storage as a stiff spring-like material during walking and running. Tendon injury is very common and debilitating, but tendon repair remains a clinical challenge for orthopaedic medicine (Wang 2006; Wang, Guo et al. 2012; Gaut and Duprez 2016)

2.1.1 Tendon structure and composition

The tendon is a multi-unit hierarchical structure composed of collagen molecules, fibrils, fibre bundles, fascicles and tendon units that run parallel to the tendon's long axis (Nourissat, Berenbaum et al. 2015) (Fig. 2.1). The basic structural unit of collagen, tropocollagen molecules, are packed together to form fibrils, the next smallest tendon structural unit. The diameter of fibrils ranges from 10 to 500 nm, depending on species, age, and sample location. These wavy crimped structures are embedded in a hydrated matrix composed of elastin and proteoglycans. Their length, demonstrated to be of the order of millimeters, is still disputable, as is whether they run continuously from muscle to bone or have ends (Ker 2007). Fibres, the next level of tendon structure, are composed of collagen fibrils and are bound by endotenons, a thin layer of connective tissue that contains blood vessels, lymphatics and nerves. Bundles of fibres form the fascicles, which are enclosed by the epitenon, which is a fine loose connective-tissue sheath containing the vascular, lymphatic and nerve supply to the tendon (Kastelic, Galeski et al. 1978; Kastelic, Palley et al. 1980). Paratenon is the third layer of connective tissue that surrounds the bundles of fascicles (synovial sheath in some sites), which in combination with

epitenon, makes up the peritenon that reduces friction with adjacent tissues (Schatzker and Branemark 1969).

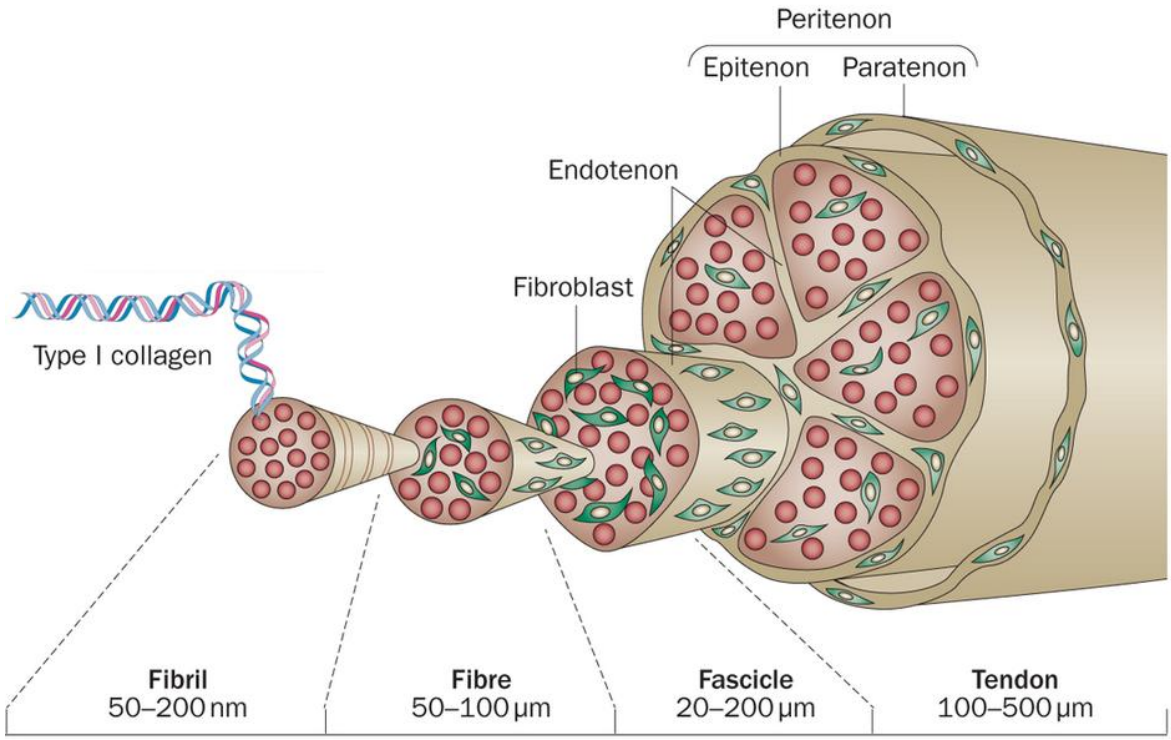


Figure 2.1. Anatomy of a normal tendon. This figure was adapted with permission from Nourissat (Nourissat, Berenbaum et al. 2015).

Tendons consist of six main components – collagens, elastin, glycoprotein, proteoglycans, water and cells. Collagen is the predominant component and makes up approximately 65 - 80% of the dry weight of tendon. Elastin is found in small quantities, comprising 1 - 2% of the total dry weight of tendon (Kannus 2000), compared with aorta which has up to 60% elastin composition (Kirkendall and Garrett 1997)). Elastin in tendon is highly associated with cell locations, but it does not contribute to tissue resilience in a manner that is similar to aorta (Fung 1993; Grant, Thompson et al. 2013). The interwoven structures of the parallel collagen fibres and elastin are stabilised by highly hydrated

proteoglycans. Their dense protein core is covalently attached to glycosaminoglycans (GAGs). There are several glycoproteins present in the extracellular matrix (ECM) of the tendon. These glycoproteins include tenascin-C and fibronectin. Tenascin-C contributes to the mechanical stability of the ECM through its interaction with collagen fibrils (Elefteriou, Exposito et al. 2001; Pas, Wyszko et al. 2006). Fibronectin is located on the surface of collagens, and its synthesis increases to facilitate tendon healing, especially at the early stage (Williams, McCullagh et al. 1984; Jozsa, Lehto et al. 1989). Tendon in vivo is composed of 70% water (Gupta, Seto et al. 2010), so hydration plays a significant role in tendon mechanics. Finally, tendon cells or tenocytes are arranged along collagen fibres and connected together by cells processes. Fibroblasts (tenoblasts and tenocytes) are the dominant cell type, but there are also endothelial cells, synovial cells and chondrocytes present in tendons (Wang 2006). Fibroblasts are responsible for synthesising ECM proteins (e.g. collagens, fibronectin and proteoglycans), producing an organised collagen matrix, and remodelling the respective ECM components during homeostasis, healing and degeneration (Wang 2006; Babaei, Davarian et al. 2016). In addition to having low metabolic activity, the oxygen consumption of tendon is 7.5 times lower than skeletal muscles (Vailas, Tipton et al. 1978). This process protects tendons against oxygen deprivation, which could otherwise lead to ischemia and necrosis, which occurs while the tissue maintains mechanical tension for long periods of time (Williams 1986; Sharma and Maffulli 2006). Anaerobic respiration is essential for tendon survival. As a consequence of having a low metabolic rate, tendon has difficulty healing following an injury (Williams, Elder et al. 2008). The slow rate of recovery is a constant challenge for clinicians; hence essential therapies are being developed to expedite the rate of healing.

Collagen fibrils provide the tendon with its strength and stiffness, as is also the case in other soft biological tissues, e.g. skin, cornea and cartilage (Fratzl 2003). Collagen

molecules in the matrix are cross-linked, which increases the Young's modulus and reduces the strain at failure (Depalle, Qin et al. 2015). Collagen type I as the most abundant component in tendons constitutes about 60% of the dry mass of the tendon and about 95% of the total collagen (Riley, Harrall et al. 1994). The remaining 5% consists of types III and V collagens. In general, type III collagen forms smaller and thinner structures (Eriksen, Pajala et al. 2002) and less organised fibrils (Lapiere, Nusgens et al. 1977; Nielsen and Karsdal 2016) which may result in decreased mechanical strength (Eriksen, Pajala et al. 2002). Type V collagen is intercalated into the core of type I collagen fibrils and regulates fibrils' growth (Birk and Trelstad 1986).

2.1.2 Tendon mechanical properties

Tensile test of tendons results in a stress-strain curve that is typical of soft fibrous tissues, see Figure 2.2 (Holzapfel 2001; Wang 2006). The initial toe region, with strains of up to 2%, represents the straightening of the initially "crimped collagen", as first observed using plane polarised light (Diamant, Keller et al. 1972). The nonlinearity depends on the type of tendon and the specimen being tested, e.g. the rat tail tendon has a smaller nonlinear region than human specimen (Kleiner 1998; Hansen, Weiss et al. 2001; Screen, Bader et al. 2004). The stress-strain response at low strains is particularly important because physiological strains are confined to this region. The slope of the linear region is often quoted as a material property, the Young's modulus. Microscopic tearing of tendon fibres occurs when the tendon is stretched over 4%. More stretching to the tendon (8 – 10%) results in macroscopic failure (Butler, Goldstein et al. 2000). Some tendons, particularly in race horses, can sustain strains of up to 14% (Devkota and Weinhold 2003). The stress-strain curve is used to quantify the mechanical and material properties. Previous studies both in animal and human have reported Young's modulus values for Achilles

tendons in tension ranging from 1 to 2 GPa (Butler, Grood et al. 1978; Shadwick 1990; Maganaris, Narici et al. 2008). The Ultimate Tensile Strength (UTS) is nearly 100 MPa and strain at failure ranges between 4 and 10% (Partington and Wood 1963; Elliott 1965; Butler, Grood et al. 1978). Another study on human patellar tendon for younger donors (aged 29 – 50 yrs) measured its ultimate tensile strength of 64.7 ± 15.0 MPa, while it was slightly lower, 53.6 ± 10.0 MPa for older donors (aged 64 – 93 yrs) (Johnson, Tramaglini et al. 1994). The strain at failure and the Young's modulus for the young and old groups was 14 ± 6% and 15 ± 5%, and 660 ± 266 MPa and 504 ± 222 MPa, respectively. Another study has estimated the in vivo structural and mechanical properties of the human tibialis anterior (TA) tendon, and the measured values of Young's modulus at maximum isometric load was 1.2 GPa (Maganaris and Paul 2002).

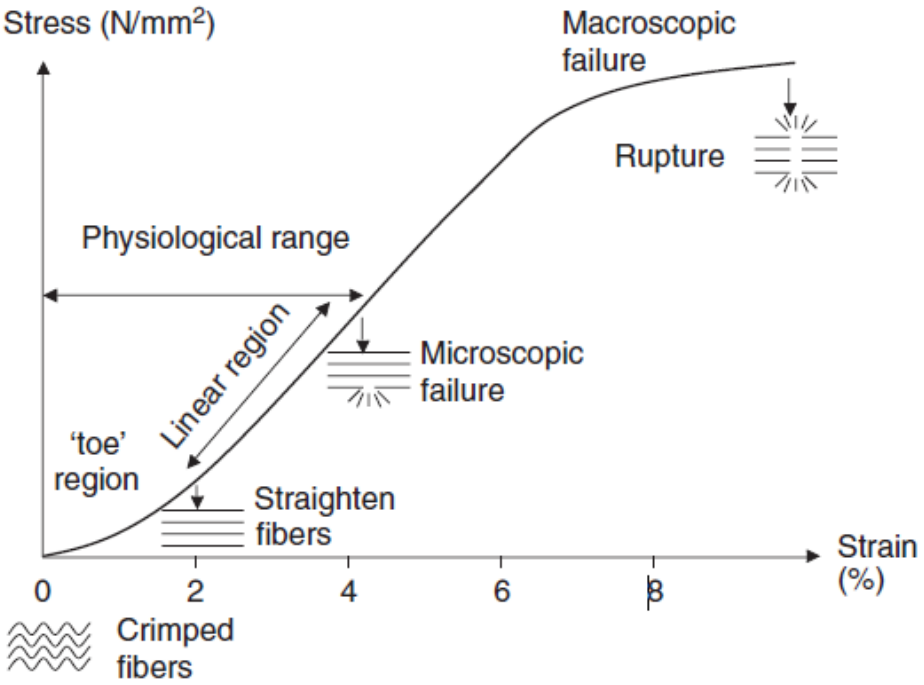


Figure 2.2. Tendon stress-strain curve. This figure was adapted with permission from Wang (Wang 2006).

2.1.3 Tendon rupture

Tendons can withstand repeatedly applied high forces, e.g. human Achilles tendon (AT) can bear load up to five times the bodyweight during running (Komi, Fukashiro et al. 1992). Despite being the strongest tendon in the body, the AT is one of the most commonly ruptured tendons (Peek, Malagelada et al. 2016). In humans, tendon disease and injury are common and costly, affecting both professional athletes (UK Achilles tendinopathy estimated incidence 500 per year (Jarvinen, Kannus et al. 2005)) and the general population (UK Achilles rupture incidence up to 20 / 100,000 per year (Lantto, Heikkinen et al. 2015)), with earnings loss and painful and prolonged rehabilitation. In equine athletes, 40% - 50% of all injuries are tendon or ligament related, with over 15% of racehorses suffering from tendon injuries during a training season. This type of injury is the most common reason for retirement of the horse (Thorpe, Clegg et al. 2010). These figures of rupture incidences have been increasing over time in any reported cohort (Maffulli, Waterston et al. 1999; Kelsall, Chapman et al. 2014).

When the tendon is torn or ruptured, patients might hear a snap or feel a sudden sharp and severe pain (Sarsilmaz, Varer et al. 2011), which usually settles quickly, although, there eventually may be some aching or no pain at all (Khan and Carey Smith 2010). After the injury, patients are normally experiencing disabling movements (Wilkins and Bisson 2012), e.g. a flat-footed type of walk and inability to stand on tiptoe, following Achilles tendon rupture. Swelling around the affected area is also common, and a sign of onset healing (Thomopoulos, Parks et al. 2015; Malagelada, Clark et al. 2016). Scar tissue after rupture, composed of disorganised collagen fibre, is frequently found and always associated with insufficient mechanical function. The decreased blood supply after rupture favours the formation of this tissue (Kraemer, Lorenzen et al. 2009).

2.2 Tendon Healing

2.2.1 The mechanism

Tendon healing can be largely divided into three overlapping phases - the inflammatory, reparative (proliferation) and remodelling (consolidation and maturation) phases (Woo, Hildebrand et al. 1999; Sharma and Maffulli 2006; Aspenberg 2007; Voleti, Buckley et al. 2012; Docheva, Müller et al. 2015) as shown in Figure 2.3. The key molecular, cellular and matrix changes during the process are as shown in Table 2.4 (Kajikawa, Morihara et al. 2007; James, Kesturu et al. 2008; Docheva, Müller et al. 2015). Each healing stage is characterised by involvement of different growth factors, activation of certain cell types and production of essential matrix proteins, which collectively contribute to the replacement of the initial fibrous tissue with more a tendonous regenerate.

Inflammatory - The initial inflammatory phase occurs almost immediately after tendon injury and lasts about 24 hours to three days (Wang 1998; Thomopoulos, Parks et al. 2015). Haematoma formation due to injury of the surrounding vascular vessels, begins the healing process (Lin, Cardenas et al. 2004). Erythrocytes, platelets, and inflammatory cells (e.g. neutrophils, monocytes, and machrophages) then migrate to the repair site and clean the site of necrotic materials by phagocytosis (Gelberman, Woo et al. 1982; Wang 1998; Wang 2006). In the meantime, macrophages aid in two functional stages: the recruitment of tendon fibroblasts to begin collagen synthesis and the release of vasoactive factors to initiate angiogenesis within the repair site (Gelberman, Chu et al. 1992; Fenwick, Hazleman et al. 2002; Wang 2006). The newly formed ECM during this phase is collectively stabilised by the increment of DNA, fibronectin, glycosaminoglycan, water and collagen type III (Montgomery 1989; Woo, Debski et al. 2000; Lin, Cardenas et al. 2004).

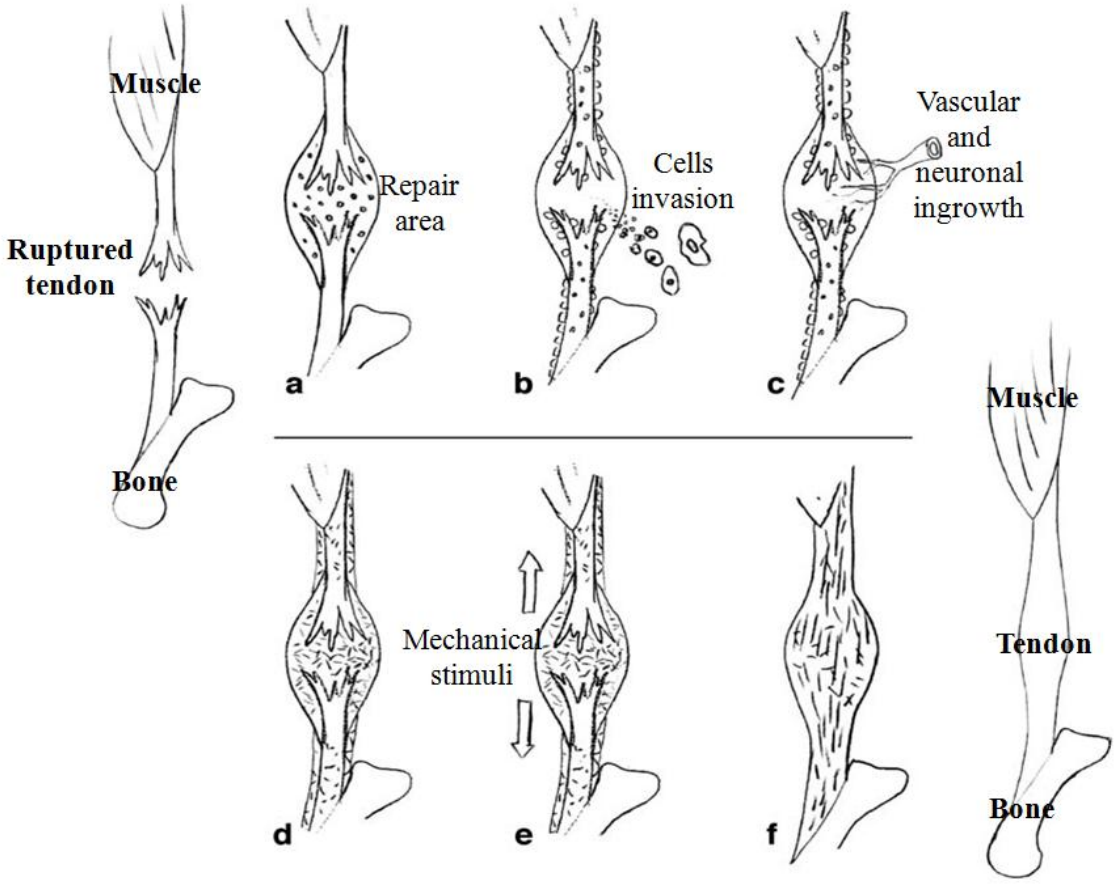


Figure 2.3. Scheme of tendon repair. Starting from inflammatory phase, which sequentially covers the haematomic condition with platelet activation (a), invasion of cells and proliferation of paratenon (b) and vascular and neuronal ingrowth (c). This phase was followed by reparative phases which involve the formation of loose collagenous callus (d) and mechanical stimulation (e). The final phases are the maturation and remodelling which primarily involves the re-organisation of collagen fibre orientations (f). This illustration was adapted with permission from Aspenberg (Aspenberg 2007).

Reparative - The reparative phase begins after a few days and last for a few weeks (Wang 1998). In this phase, tendon fibroblasts synthesise an abundance of collagen randomly and other ECM components such as proteoglycans, which are then deposited at the repair site (Józsa and Kannus 1997; Wang 1998). Type III collagen and DNA

concentrations reach their peak amounts during the entire reparative process, and are thus believed to contribute to the optimisation of collagen synthesis and the gradual conversion of type III to type I collagen (Woo and Buckwalter 1988; Aspenberg 2007; Oakes 2008). Water content and glycosaminoglycan concentration remain high. Subsequently, the size of the repair site is increased in order to provide a better mechanical environment for sustaining considerable traction forces during the healing process (Woo, Hildebrand et al. 1999; Wang 2006; Aspenberg 2007; Eliasson, Andersson et al. 2009).

Table 2.4 Key molecular, cellular and matrix changes occurring during the three main phases of tendon repair. With permission from (Kajikawa, Morihara et al. 2007; James, Kesturu et al. 2008; Docheva, Müller et al. 2015)

	Inflammatory	Reparative (proliferation)	Remodelling (consolidation & maturation)
Cells & Matrix Changes	Platelets Neutrophils Monocytes Erythocytes Circulation-derived mesenchymal stem cells	Cellularity and Matrix production Collagen type III Activation of local tendon stem/ progenitor cells	Cellularity and Matrix production ⇩ Collagen type III ⇩ Collagen type I ⇧
Molecular Changes	Interleukin-6, -1β bFGF IGF-1 PDGF TGFβ VEGF	bFGF GDF-5, -6, and -7 IGF-1 PDGF TGFβ VEGF	GDF-5, -6, and -7 IGF-1 TGFβ

Remodelling - The remodelling phase follows after about 4 to 6 weeks, and lasts up to 18 weeks and can be divided into a consolidation stage and a maturation stage (Tillman and Chasan 1996; Lin, Cardenas et al. 2004; Sharma and Maffulli 2005). During consolidation, cellularity, fibroblast size and collagen decrease (Wang 1998; Wang 2006). The scar tissue changes colour from red to pinkish and translucent, and connects the two

ends of the ruptured tendon (Lin, Cardenas et al. 2004; Sasaki, Yamamoto et al. 2012). By six weeks the gap site is filled with collagen which gradually reorients along the loading direction (Hooley and Cohen 1979; Wang 1998; Wang 2006; Sasaki, Yamamoto et al. 2012). The fibroblasts start to become inactive tenocytes (Wang 1998). Covalent bonding between collagen fibres increases, starting from week eight, which results in repaired tissue with higher stiffness and tensile strength (Gelberman, Woo et al. 1982). Although the tensile strength as the healing tendon improves, it will not achieve the same levels as uninjured, normal tissues (Lin, Cardenas et al. 2004). After ten weeks, the maturation stage occurs, with gradual change of the fibrous tissue to scar-like tendon tissue over the course of one year (Farkas, McCain et al. 1973; Hooley and Cohen 1979). Metabolism of tenocytes and tendon vascularity also decline (Amiel, Akeson et al. 1983; Wang 2006). Maturation of the scar involves replacing type III collagen with type I, increasing collagen crosslinks glycosaminoglycan, water, and DNA concentrations as well as callus size (Abrahamsson 1991; Aspenberg 2007; Eliasson, Andersson et al. 2009).

2.2.2 Intrinsic and extrinsic healing

Two forms of tendon healing have been identified – intrinsic and extrinsic (Wang 1998; Lin, Cardenas et al. 2004; Sharma and Maffulli 2005; Sharma and Maffulli 2006). The intrinsic healing is associated with two factors – resident epitenon and endotenon tenocytes, and the intratendinous blood supply (Lundborg 1976; Lundborg and Rank 1978; Manske, Gelberman et al. 1984; Manske 1988). Epitenon fibroblasts initiate the repair process through proliferation and migration, and they are the first cell type to synthesise collagen, followed later by endotenon fibroblasts (Ingraham, Hauck et al. 2003). Tenocyte functions may vary depending on the region of origin (Riederer-Henderson, Gauger et al. 1983). For instance, more collagen and GAG is produced by cells that originate from

epitenon and endotenon than from tendon sheath or paratenon. Additionally, the intrinsic healing process causes less formation of adhesions between the tendon and surrounding tissue (Manske, Gelberman et al. 1984; Manske 1988; Mass and Tuel 1989; Mass and Tuel 1991) leading to greater tensile strength and better gliding function (Gelberman, Vande Berg et al. 1983; Manske, Gelberman et al. 1984; Mass and Tuel 1989).

Extrinsic healing is mediated by processes that originate outside of the tendon in the surrounding sheath and synovium. Extrinsic healing is associated with adhesions which are needed for assisting the ingrowths of fibroblasts, inflammatory cells and extratendinous blood vessels (Potenza 1962; Potenza 1963; Potenza 1964; Potenza 1969; Manske and Lesker 1984). In extrinsic healing adhesions are important for vascularisation and the cellular process of the healing, but reduce the mobility of the healed tendon (Wang 1998; Sharma and Maffulli 2006).

Despite poor evidence as to which theory is correct, it is most likely that tendon healing is a combination of both processes. Typically, the extrinsic occurs earlier followed by the intrinsic (Butler, Juncosa et al. 2004; Docheva, Müller et al. 2015). It is also suggested that these processes are dependent on tendon location, the magnitude of tendon trauma, availability of synovial fluid and a blood supply, repair technique and degree of tendon mobilisation (Wang 1998; Lin, Cardenas et al. 2004).

2.2.3 Material properties and structural change

A sequence of material properties and structural alterations occurs during healing. This has been studied in both animal models of tendon healing and also in humans. A study of rabbit Achilles tendon healing following complete transection showed that the Young's moduli of the healing tendons were approximately five times lower than control three weeks after surgery, increasing to nearly 80% at 12 weeks (Nagasawa, Noguchi et al.

2008). A similar pattern was observed with tensile strength. In contrast, the cross-sectional area and viscoelastic phase angle were 3 and 1.5 times greater, respectively, than controls at three-weeks and declined nearly equivalent to controls at 12 weeks. A rat supraspinatus tendon-defect injury model (incomplete transection) was reported to be less than 10% of the uninjured modulus at 12 weeks post-surgery while also being incomplete in terms of restoration of collagen organisation (Carpenter, Thomopoulos et al. 1998). In a study of human Achilles tendons following rupture using a stereo X-ray technique to measure stiffness, an inverse relationship between material properties and callus dimension was found, suggesting that a compensation mechanism was regulating the healing process (Fig. 2.5) (Schepull, Kvist et al. 2007). In a rat healing study following Achilles tendon complete transection, the ultimate tensile stress and modulus tended to decrease at day 21 from day 14 despite the increasing size of the callus, suggesting that the healing process maybe driven by improving tissue energy absorption capacity, rather than making the matrix stronger and stiffer (Eliasson, Andersson et al. 2009).

Since collagen has been identified as the main load-bearing component (Lanir 1983; Szczesny and Elliott 2014), collagen fibre re-organisation is important in determining the mechanical and material properties of healing tendons (Ottani, Raspanti et al. 2001; Hulmes 2002; Connizzo, Yannascoli et al. 2013). A linear correlation was found between a validated histological score that included collagen fibre organisations and the biomechanical characteristics of healing tendons (Rosenbaum, Wicker et al. 2010). A study by Sasaki (Sasaki, Yamamoto et al. 2012) used scanning electron microscopy to visualise the three-dimensional network of the fibres in rat Achilles tendon during healing. This study found that the rate of re-orientation of initially disorganised fibres into the axial direction, termed axialisation, varied with location (Fig. 2.6). Near the tendon stump

axialisation began in the outer layer and progressed towards the core region. In contrast, the axialisation in the middle of the repair site progressed from the core to the outer layer.

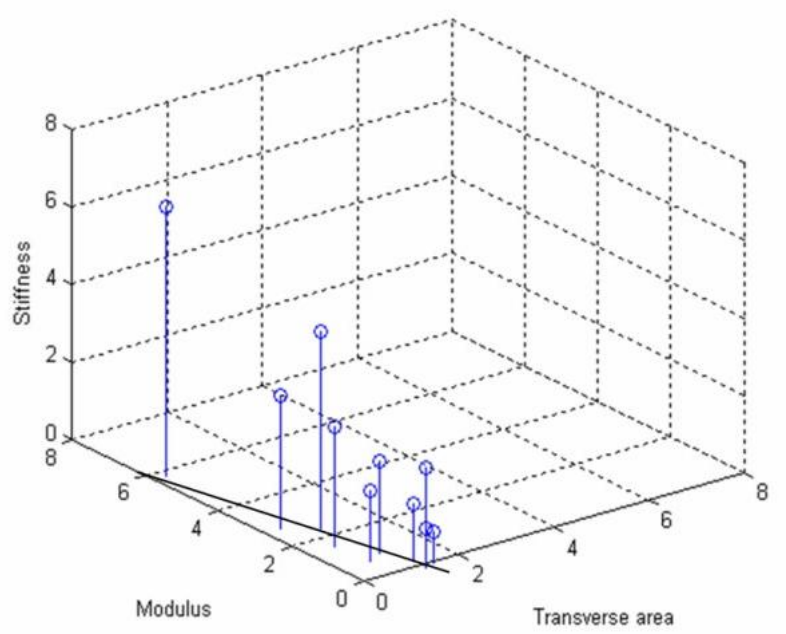


Figure 2.5. The relationships between stiffness, transverse area and modulus of healing tendons addressed by Schepull (Schepull, Kvist et al. 2007), who suggested tissue organisation is more important in the regenerated tendon than its strength and stiffness as this could better restore the physiological spring-like behaviour. Increase in stiffness is mostly caused by increasing modulus, but there is a negative correlation between increase in modulus and transverse area (symbolised by drawn line on bottom plane). This figure was adapted with permission from Schepull (Schepull, Kvist et al. 2007).

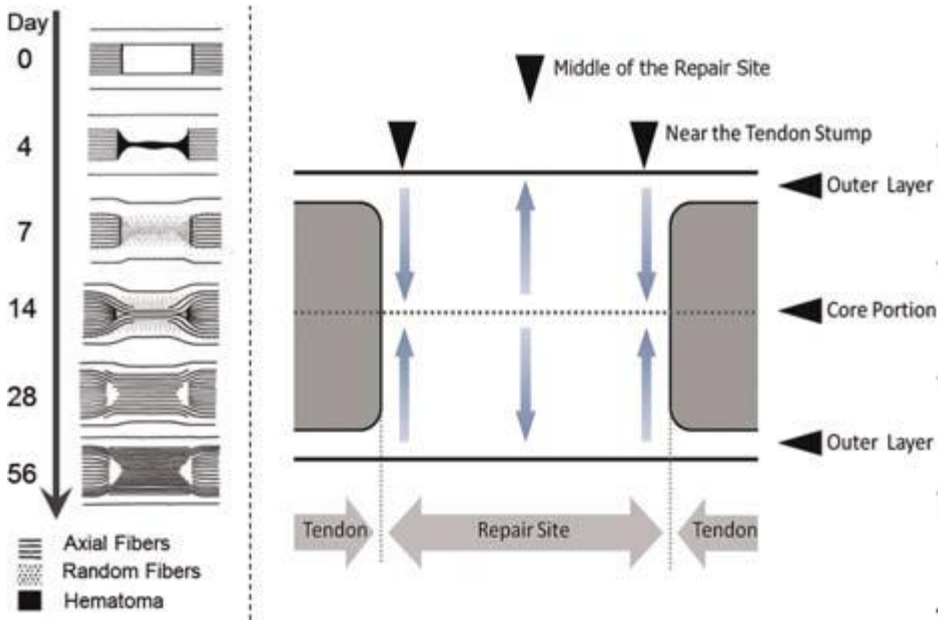


Figure 2.6. The trends of axialisation in the middle of the repair site and near the tendon stump, were completely opposite (Sasaki, Yamamoto et al. 2012). This study is particularly important to inform researchers about the spatial distribution of fibre structures across healing tissues over time, which is imperative to better understand their mechano-structural behaviours specifically at the tissue level. This figure was adapted with permission from Sasaki (Sasaki, Yamamoto et al. 2012).s

2.2.4 Tendon healing mechanobiology

Not only is tendon exquisitely designed to provide superb properties for its specific use, like many other mechanically loaded tissues, a tendon is able to adapt to withstand changing loading conditions. Mechanobiology seeks to define the precise relationship between mechanical stimulation and cellular and tissue mechanisms which underlie the observed changes in metabolism, structure and mechanical properties (Wang 2006). The experimental evidence for tendon's being mechanoresponsive has been recently reviewed (Thompson 2013; Lavagnino, Wall et al. 2015).

Many previous works have examined the effect of loading on tendons during development and homeostasis (Banes, Horesovsky et al. 1999; Thomopoulos, Fomovsky

et al. 2005; Wang 2006; Magnusson, Narici et al. 2008; Magnusson, Langberg et al. 2010; Thomopoulos, Das et al. 2011) as well as during regeneration (Sharma and Maffulli 2006; Eliasson, Andersson et al. 2009; Schepull, Kvist et al. 2012; Schepull and Aspenberg 2013; Schepull and Aspenberg 2013). Tendon microstructure and strength during prenatal and postnatal growth have been shown to require mechanical cues for their normal development (Evanko and Vogel 1993; Thomopoulos, Kim et al. 2007; Kim, Galatz et al. 2010; Thomopoulos, Genin et al. 2010). Embryonic immobalisation leads to a decrease in tenascin expression (important for regulating cell proliferation and migration) and protein levels in avian synovial joints (Mikic, Wong et al. 2000). Hence, it impairs the development of the tendon. Also in neonates, muscle paralysis caused delay in tendon and fibrocartilage maturation as well as impaired mineralisation at the enthesis (Thomopoulos, Kim et al. 2007; Kim, Galatz et al. 2010).

As for healing, mechanical loading could either be beneficial or detrimental, depending mainly on its magnitude, direction and frequency (Killian, Cavinatto et al. 2012). However, it has been generally accepted that mobilisation after repair resulted in improved tensile properties of healing tendons; particularly in promoting enhanced gliding function (Piper and Whiteside 1980; Woo, Gomez et al. 1981; Gelberman, Vande Berg et al. 1983). Gelberman (Gelberman, Vande Berg et al. 1983; Gelberman and Woo 1989) demonstrated that mobilised canine tendons healed stronger, showed better excursion and favoured smooth gliding between collagen fibres with the non-existence of adhesions at 42 days, as intrinsic healing predominated. In contrast, immobilised tendons favoured adhesion formation as extrinsic healing predominated (Fig. 2.7). In the case of canine flexor digitorum profundus tendons injured by surgical laceration, active mobilisation increased its ultimate strength compared with that of the immobilised tendon, with the force at which the repair failed was 61.6 N and 41.0 N, respectively (Wada, Kubota et al.

2001). Fibroblast activity is influenced by mechanical loading, with e.g. the upregulation of collagen synthesis in loaded healing tendons (Eliasson, Andersson et al. 2009). It is also suggested that soft tissue mobilisation promoted the healing of rat Achilles tendon after collagenase induced injury through fibroblast proliferation and collagen realignment (Davidson, Ganion et al. 1997).

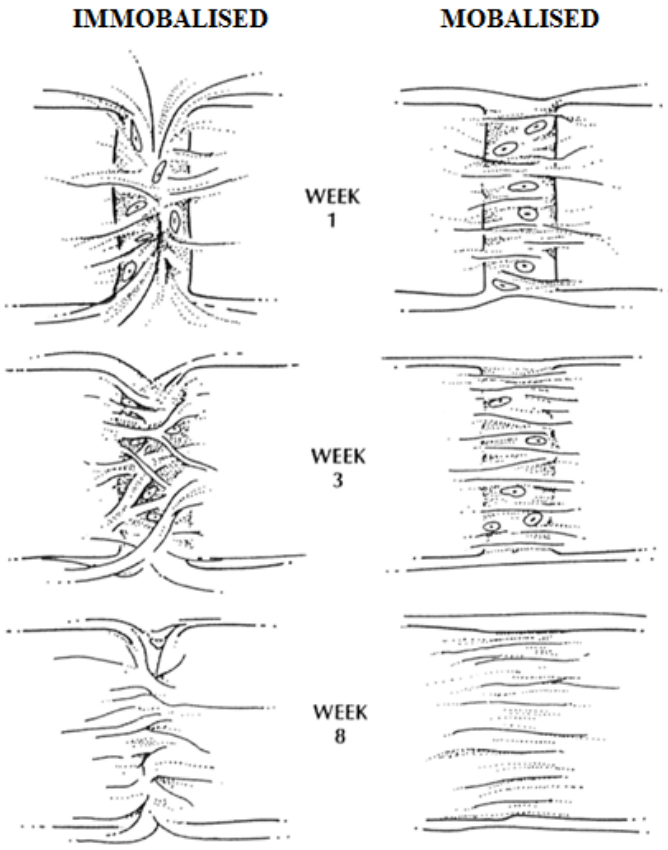
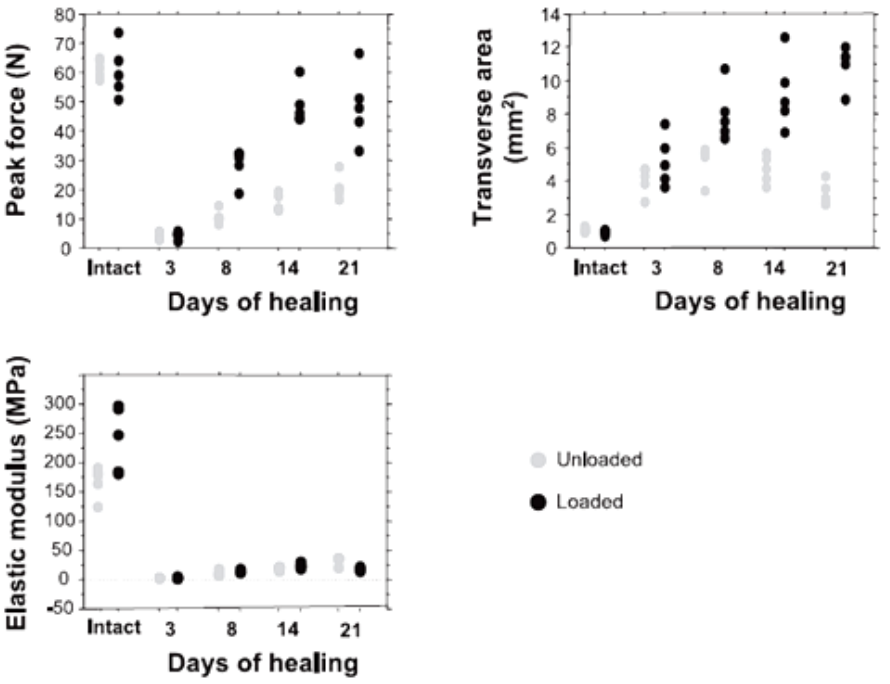


Figure 2.7. Forces applied during mobilisation restored the mechanical strength and overall healing of a repaired tendon. The initially haphazard orientation of collagen in healing tendon remains random through the remodelling phase of healing leading to adhesion formation and abnormal physiological behaviour. In contrast mobilised tendon collagen fibrils showed greater axial alignment. This figure was adapted with permission from Wang (Wang 1998)

Previous studies have shown that these positive impacts of loading are highly time dependent. For instance, a study investigating the effect of loading on the mechanical properties and gene expression of rat Achilles tendons healing by Eliasson (Eliasson, Andersson et al. 2009), found that the loading regimes applied during the early phase (in rats day 3 and 8 after transection) seemed to suppress genes related to inflammation and ECM components. This finding supported clinical experience that freshly wounded tissues should be immobilised as early inflammation might stimulate rather than impair healing. In the later phase of healing (rat Achilles day 14 and 21 after transection) loading promoted higher expression of ECM-related and tendon-specific genes, apparently supporting the transformation of the scar tissue to tendon (Fig. 2.8).



(a)

	Loaded Tendons Compared With Unloaded				
	Intact	*Day 3*	*Day 8*	*Day 14*	*Day 21*
Procollagen I		↓	↓	↑	↑
Procollagen III	↑	↓	↓		
Tenascin-C	↑				↑
Tenomodulin					↑
Scleraxis			↓	↑	↑

(b)

Figure 2.8. Difference in terms of mechanical properties (a) and gene expression (b) between loaded and unloaded tendons. ↑ means higher expression in loaded tendons compared with the unloaded. ↓ means lower expression in the loaded tendons compared with unloaded. This figure was adapted with permission from (Eliasson, Andersson et al. 2009)

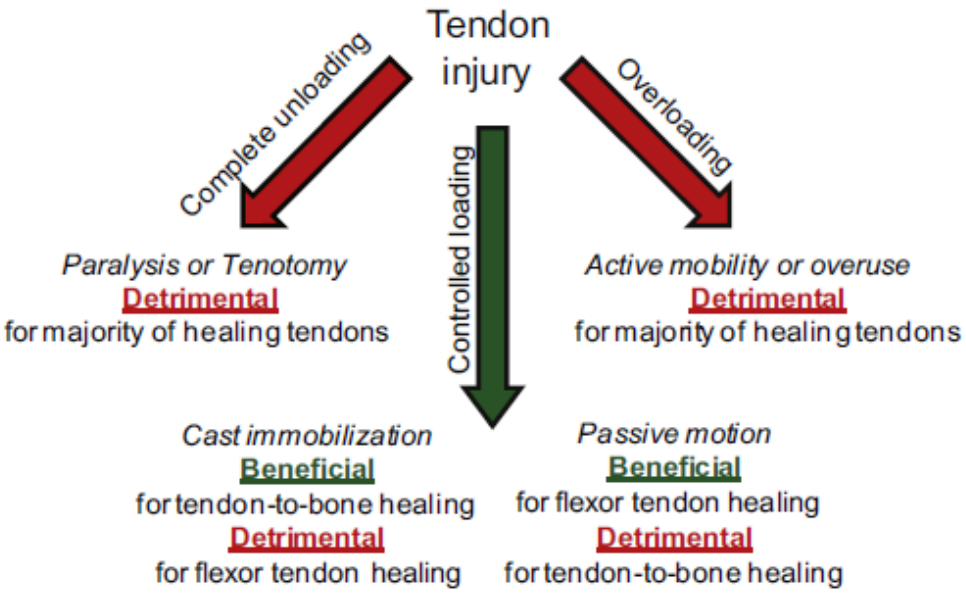


Figure 2.9. The effect of loading to healing tendons. This figure was adapted with permission from Killian (Killian, Cavinatto et al. 2012)

The aforementioned studies suggested that, care must be taken in the application of load for enhanced tendon healing. On top of making sure of the right timing to impose mechanical loading, Killian (Killian, Cavinatto et al. 2012) highlighted that a fine balance must be kept between under-stimulating and overloading healing tendons, with differences between healing at the tendon-to-bone interface and healing of flexor tendons (Fig. 2.9). Complete removal of load is detrimental to tendon healing (Uchida, Tohyama et al. 2005; Thomopoulos, Zampiakis et al. 2008; Galatz, Charlton et al. 2009), while large forces are also harmful (Soslowsky, Thomopoulos et al. 2000; Huang, Perry et al. 2004; Perry, McIlhenny et al. 2005; Glazebrook, Wright et al. 2008). Control loading, also known as passive motion, can enhance healing in most settings (Gelberman, Amifl et al. 1981; Gelberman, Woo et al. 1982; Gelberman, Manske et al. 1986; Boyer, Goldfarb et al. 2005; Schepull and Aspenberg 2013); however, a fine balance must be reached between loads that are too low (leading to a catabolic state) and too high (leading to microdamage).

2.2.5 Mechanosensing and tenogenesis

Tenocytes actively sense and process mechanical information that is provided by the extracellular environment to make decisions about growth, motility and differentiation (Wall, Dyment et al. 2016). A recent study has shown that the mechanosensing in tenocytes is regulated by Gtf2ird1-dependent Mohawk (Mkx) expression (Kayama, Mori et al. 2016). Mkx, a tendon-specific transcription factor, is important in both development and maturation into adulthood (Shukunami, Takimoto et al. 2006; Ito, Toriuchi et al. 2010; Liu, Watson et al. 2010). In a way, its absence results in hypoplastic tendons with decreased collagen fibre density (Ito, Toriuchi et al. 2010; Nakahara, Hasegawa et al. 2013; Onizuka, Ito et al. 2014). The alteration of gene expressions by tenocytes in response to mechanical stimuli is important as the mechanism results in contractile cell forces that are vital for healing process and wound closure (Grinnell 1994; Eliasson, Andersson et al. 2009). This alteration modifies production of ECM proteins resulting in adaptive changes of the tissue mechanical properties. The conversion of mechanical signal to biochemical cues which leads to cascades of cellular and molecular events that eventually lead to changes in tendon structure, known as mechanotransduction, needs to be further delineated. Mechanotransduction mechanisms involve several cellular components, primarily ECM, cytoskeleton and integrins (Fig. 2.10) (Schiele, Marturano et al. 2013).

As primary load bearing components in tendons, collagen fibres are imperative towards mechanotransduction. Fibre deformation transmits a mechanical signal to the cell's actin cytoskeleton (Wang 2000; Wang, Goldschmidt-Clermont et al. 2001), which leads to regulation of cell shape, affects cell motility and mediates various cellular functions, including DNA and protein synthesis (Janmey 1991). The components of the cytoskeleton - microfilaments, microtubules, and intermediate filaments - are responsible for responding to extracellular forces, participate in transmembrane signalling, and provide

a network for translocating signalling molecules. Mechanical forces applied to the cell surface transmit directly to the cytoskeleton and cause changes in its structure (Wang and Ingber 1995). Consequently, these changes initiate transduction cascades within the cells, e.g. gene expression alteration (Iwasaki, Eguchi et al. 2000; Lehoux and Tedgui 2003; Schepull, Kvist et al. 2007).

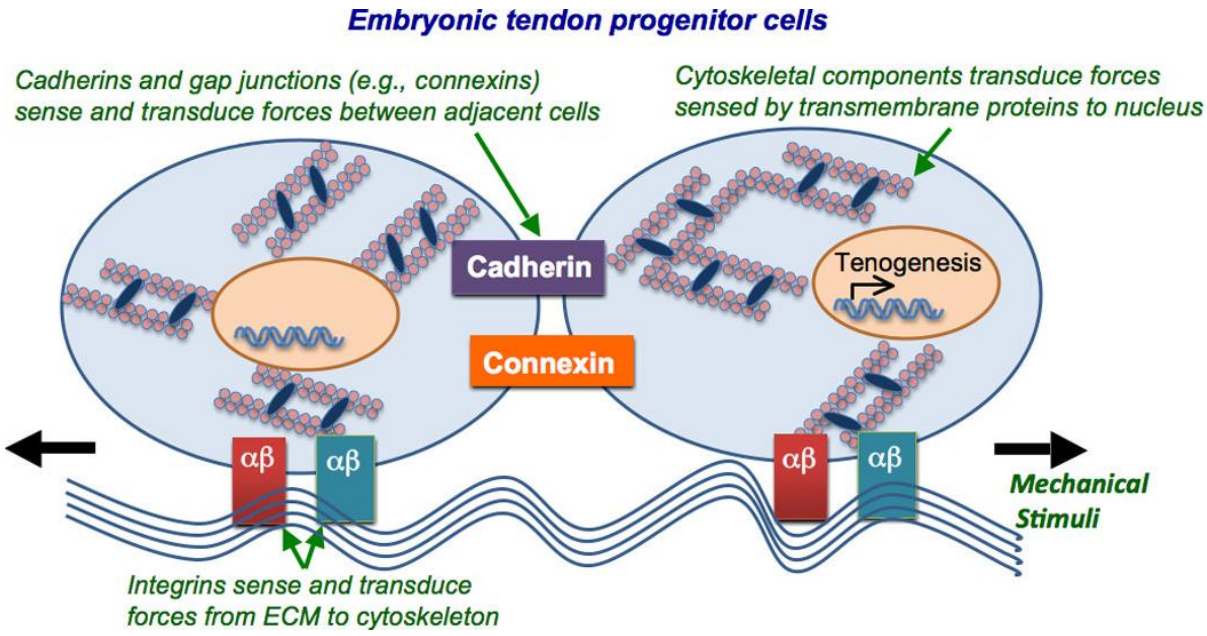


Figure 2.10. Mechanotransductive components of tendon stem cells. The cells may sense and transduce mechanical signals between cells via direct cell-to-cell contacts such as cadherin (purple) and connexin (orange), and from the surrounding ECM via integrins (red and blue). Downstream, cytoskeletal components that link to these transmembrane proteins may transduce forces to the nucleus to regulate gene and protein expression. This figure was adapted with permission from Schiele (Schiele, Marturano et al. 2013)

Generation of tendon tissue, tenogenesis, under controlled mechanical loading conditions has been studied mainly in embryonic stem cells (McBride, Trelstad et al. 1988; Nakagaki, Biancalana et al. 2007; Herchenhan, Kalson et al. 2011; Marturano, Arena et al.

2013; Schiele, Marturano et al. 2013; Kalson, Lu et al. 2015). The influence of dynamic tensile loading on tenogenic gene expression has been addressed in an in vitro study (Schiele, Marturano et al. 2013) (Fig. 2.11). Upregulated expression of scleraxis, a tenogenic gene, was found in murine mesenchymal stem cells (MSCs) undergoing cyclic strain in collagen gels (Scott, Danielson et al. 2011). Studies on human MSCs in response to cyclic strain have found maintained or upregulated expression of tenogenic genes (scleraxis, collagen types I and III, tenascin-C) and increased matrix production (Kuo and Tuan 2008; Xu, Song et al. 2011). Substrate stiffness may also mediate tenogenesis as stem cells on a collagen-coated substrate with elastic modulus of 40 kPa showed increased scleraxis, tenomodulin, tenascin-C and collagen III gene expression in bone marrow stromal stem cells, relative to 20 kPa and 80 kPa (Sharma and Snedeker 2010) (Fig. 2.11). Elastic modulus may be an important cue for tenogenesis by adult stem cells and deserves further investigation. Different effects of compression and tension on MSCs in collagen constructs were examined, which found that tensile loading favours tenogenic while compressive loading enhances fibro-cartilage-like phenotype (Thomopoulos, Das et al. 2011). However, this study also highlighted that the underlying mechanism, e.g. how the tendon progenitor cells sense and respond differentially to tensile and compressive loads, remains unclear.

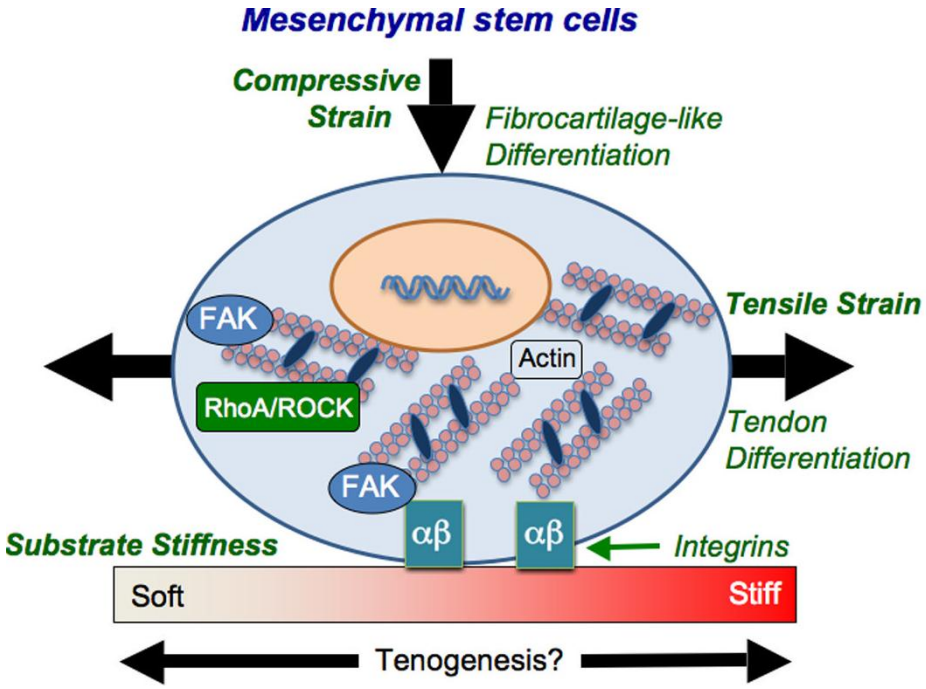


Figure 2.11. Mechanical factors may influence tenogenic differentiation of mesenchymal stem cells. The tenogenic effect of dynamic tensile strain may be mediated through FAK, RhoA/ROCK and the actin cytoskeleton in MSCs. While tension is tenogenic, compressive loading may induce a fibrocartilage-like phenotype. Substrate stiffness may provide an additional mechanical signal to influence stem cell tenogenesis. This figure was adapted with permission from Schiele (Schiele, Marturano et al. 2013)

2.3 Biological strategies to augment tendon healing

Finding the right approach for treatment of ruptured tendon has been challenging, in particular to re-establish the continuity of collagen fibres and restore the sliding surface of tendon. The aforementioned behaviours of healing tendons, particularly the mechano-responsiveness with respect to stem cell differentiation and ECM synthesis, may inspire the development of future treatments.

A recent review by Docheva (Docheva, Müller et al. 2015) discussed current strategies to augment tendon repair – growth factors, stem cells, biomaterials and gene therapy. Tendon injury stimulates the production of a variety of growth factors at multiple

stages in the healing process (Evans 1999; Yang, Rothrauff et al. 2013) leading to increased cellularity and tissue volume (Sharma and Maffulli 2006). To name a few, *bFGF* (important to promote early events in the healing) (Würgler-Hauri, Dourte et al. 2007; Heisterbach, Todorov et al. 2012), BMP (important in establishment of tenogenic factors with the potential of driving differentiation of MSC in vitro (Park, Hogan et al. 2010) and in vivo (Wolfman, Hattersley et al. 1997), VEGF (to promote angiogenesis which is important in regeneration). Growth factors can be applied by local injection percutaneously or operatively, or by implanting scaffolds or even suture materials (Rickert, Jung et al. 2001; Hamada, Katoh et al. 2006; Uggen, Dines et al. 2010) containing growth factors.

Stem cells are suggested to be used either alone or in combination with biocompatible scaffolds, which are delivered intra-operatively to the site of the tissue damage (Lui 2015). This technique is claimed to be one of the most attractive and widely explored approaches for musculoskeletal regeneration. There are several cell types with regards to tissue engineering tendons, including MSCs from different tissue sources (bone marrow (BM), adipose tissue (AD), embryonic stem cells (ECSs) to name a few (reviewed in (Obaid and Connell 2010; Longo, Lamberti et al. 2011; Ahmad, Wardale et al. 2012)).

Biomaterials have been explored as alternatives to tendon transplant (autologous or allogeneic) to resolve the problem of donor site morbidity, risk of immune rejection, and material properties mismatched (reviewed in (Longo, Lamberti et al. 2010; Zhang, Xu et al. 2012). An ideal biomaterial for treatment of ruptured tendon should exhibit these criteria: (1) biocompatibility, (2) support cell attachment and growth, (3) high surface area, (4) promote tenogenic differentiation pathway, (5) not inducing host inflammatory responses, (6) mimic native tendon architecture and mechanical properties, and (7) reproducible, scalable, good storage properties and customisable when it comes to scaffold

(Docheva, Müller et al. 2015). Most biomaterials studies have investigated how MSCs or tendon-derived cells respond to these materials in terms of cell adhesion, cell proliferation and survival over time, gene expression and differentiation (Longo, Lamberti et al. 2010; Longo, Lamberti et al. 2012; Zhang, Bogdanowicz et al. 2012). One collagen-based material loaded with bone marrow MSCs used to repair different tendon gap models, provided greater structural support and cell viability to the healing tissue (Young, Butler et al. 1998; Awad, Boivin et al. 2003; Juncosa-Melvin, Shearn et al. 2006), which help promote tenogenesis (see Section 2.2.5). Cell-loaded nanobiomimetic scaffolds with aligned collagen I structure (e.g. nanofibres and nanocomposite) convincingly showed, in vitro, that their aligned topography can affect a cell morphology in a manner similar to that of tenocytes, thus promoting aligned matrix reproduction that favours the upregulation of tendon-related genes, e.g. scleraxis and collagen type XIV (Alfredo Uquillas, Kishore et al. 2012; Kishore, Bullock et al. 2012). These studies also suggested that aligned nanofibres could be superior to randomly oriented biomaterials by promoting tenogenic differentiation of the implanted cells. However, rescaling to dimensions relevant for the repair of human tendon has still been a challenge.

Gene therapy has the potential to promote the repair mainly by delivering biological factors to sites of injury (Evans and Robbins 1995; Evans and Robbins 1999). In particular, it allows the local, focal production of gene products within and around the lesion in a sustained and potentially regulated fashion, and promotes greater biological activity with a minimum risk of triggering neutralising immune reactions.

3 Mechanobiological modelling of tendons: review and future opportunities

This chapter reviews and addresses future opportunities of mechanobiological modelling of tendons. Investigations performed in the past have focused on different levels of this multi-hierarchical tissue, i.e. tissue, fibrillar, cellular and combination of levels (multi-level). However most of the works were performed to understand the tissue's biomechanical behaviours. So far only simple analytical approaches have been applied to tendon mechanobiology. If the development of sophisticated computational mechanobiological models is conducted in parallel with reporting more quantitative in vivo mechanobiological data, then this field offers huge potential for future development towards clinical applications.

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3.1 Introduction

Understanding healthy and pathological mechanisms for tendon adaptation to mechanical loading is key to developing novel therapies that harness the positive mechanoresponse of the tissue, independent of whether they are pharmacological, mechanical or tissue engineering-based. Moreover, since tendons contain high levels of collagen type I, tendon can act as a simple model system for other more complex, less well-studied collagen based tissues. Finally, the knowledge can also assist in biomimetic design of new highly fatigue resistant, self-healing composite materials.

Precise mechanisms for the tendon adaptive response to mechanical load remain unknown. For tissue adaptation driven by cell activity, understanding the mechanical environment of the living cells and hence the tissue micromechanics are essential pre-requisites. Cells are believed to sense multiple physical signals from the environment through mechanisms sensitive to substrate deformation and fluid movement (Fig. 3.1) and respond anabolically or catabolically. Computational modelling can address these challenges with both 'biomechanical models' (see e.g. Chapter 4) that provide a detailed quasi-static picture of the biomechanics of the system at a given time, often focusing on the material model and properties of the tissue, and also with 'adaptive models' (see e.g. Chapter 5) that represent the ability of the tissue to remodel over time, predicting the long term mechanobiological adaptation, often trying to identify the cell and tissue response to certain stimuli. The aim of this chapter is to provide an overview of the latest developments in computational modelling that can be applied to understanding and harnessing tendon biomechanics and mechanobiology and to set out the future directions in this field.

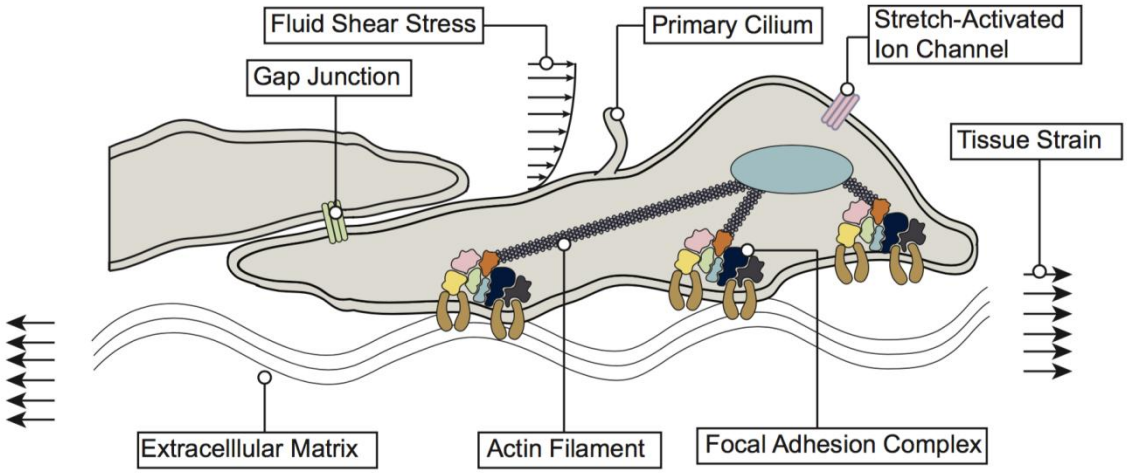

Figure 3.1. Mechanotransduction mechanisms in tendon. The tendon cell, connected mechanically to its pericellular matrix and to adjacent cells, transduces stretch and fluid shear stress signals through cytoskeletal, membrane and primary cilium mechanisms. With permission (Grant 2014).

Following recent discussion of nomenclature (Handsfield, Slane et al. 2016), this review uses the term fibril to refer to structures consisting of quasi-crystalline collagen which may be up to 500 nm in diameter. The definition of larger structures may be specific to certain tissues. In tendons, fibres are bundles of collagen fibrils that may contain other proteins and cellular components.

3.2 Biomechanical models

3.2.1 Whole tendon models

Whole tendon modelling is essential to define the macro-scale mechanical environment and response of the tissue in biomechanical analysis of locomotion. The mechanical properties of tendons are largely dependent on the way in which the fibril organisation changes with loading (stress-strain curve in Fig. 2.2 of the previous chapter).

The simplest phenomenological models for the non-linear tendon stress-strain behaviour have used various strain energy functions including exponential (Fung 1967), polynomial (Vaishnav, Young et al. 1973) and logarithmic (Takamizawa and Hayashi 1987). Uniform isotropic hyperelastic models have also been used extensively (Veronda and Westmann 1970; Hirokawa and Tsuruno 1997; Hirokawa and Tsuruno 2000). However, as is clear from the previous chapter the strongly anisotropic fibril orientation and the clear role fibril re-orientation plays in tendon motivate a more sophisticated approach.

Microstructurally motivated continuum approaches have used transversely isotropic hyperelasticity with a single set of fibril orientations to represent tendon and ligament (Weiss, Maker et al. 1996) and variants have been used in many finite element implementations for example in supraspinatus (Zobitz, Luo et al. 2000) and the long head of the biceps tendon (Carpenter, Wening et al. 2005; Hwang, Carpenter et al. 2014). Subsequent developments included modelling two distinct families of collagen fibres in cruciate ligaments (Hirokawa and Tsuruno 2000), and most recently patient-specific Achilles models with realistic geometries (Shim, Fernandez et al. 2014). A similar early model for the arterial wall (Holzapfel, Eberlein et al. 1996; Holzapfel and Gasser 2001) has also evolved, incorporating two fibre families each with a symmetric dispersion about their mean orientation angle (Gasser, Ogden et al. 2006), which has been successfully applied with a design of experiments approach to represent the mechanical response at discrete timepoints of tendons undergoing healing (Bajuri, Isaksson et al. 2016) (see Chapter 4 for more details). These models have also been modified to include a rule-of-mixtures fibre volume fraction (Driessen, Cox et al. 2008).

Fibril recruitment due to the straightening of crimp has long been considered in models of soft tissue (Frisén, Mägi et al. 1969) and continuum models have been proposed

with various distribution functions used to represent the recruitment stretch: Weibull (Hurschler, Loitz-Ramage et al. 1997), Gamma (Szczesny, Peloquin et al. 2012), or triangular function (Watton, Hill et al. 2004; Aparício, Thompson et al. 2016). Most recently a continuum strain energy function was proposed (Shearer 2015) with a recruitment function derived from considerations of the radial distribution of crimp angle (Kastelic, Palley et al. 1980), with good fitting results to patellar tendon data. This strain energy function was further refined to represent the helical arrangement of crimped fibrils although without representation of mechanical interactions between fibrils (Shearer 2015).

The continuum approach has further been used to identify the importance of mechanical interactions between fibrils and the matrix in which they are embedded to accurately represent biaxial testing of tendons (Guerin and Elliott 2004). Further work has suggested that this mechanism for transmission of interfibrillar shear stress is the primary loading mechanism for collagen fibrils in tendon (Szczesny and Elliott 2014). Another fibre reinforced anisotropic hyperelastic constitutive model has been implemented to study the effect of partial-thickness tear on loading capacities of the supraspinatus tendon (Engelhardt, Ingram et al. 2015). The widespread assumption of incompressibility in soft tissue has been challenged in ligaments (Weiss, Gardiner et al. 2005), and recent models with slight compressibility have been implemented in a finite element analysis (Pierrat, Murphy et al. 2015).

Viscoelastic models have been developed to capture the time dependent character of tendon behaviour. The most popular, Quasi Linear Viscoelasticity (QLV) uses a hereditary integral formulation often with the exponential strain energy function mentioned earlier (Fung 1968; Haut and Little 1972; Woo, Gomez et al. 1981; Woo 1982; Johnson, Tramaglini et al. 1994; Lucas, Bass et al. 2008). This approach has been developed to implement Mooney Rivlin or Prony series constitutive laws for further

efficiency and better fitting at high strains in stress relaxation (DeFrate and Li 2006; Tang, Ng et al. 2011). Fully non-linear viscoelasticity has also been applied to capture a wider range of behaviours under physiologically relevant loading (Duenwald, Vanderby et al. 2010; Troyer, Shetye et al. 2012). Some structurally motivated continuum models including viscoelastic behaviour have been proposed for tendons, where different constitutive equations for different levels of strain have been "patched" together (Johnson, Livesay et al. 1996) or using QLV to separately represent the isotropic and anisotropic components of the tissue (Vena, Gastaldi et al. 2005). X-ray diffraction studies motivated a model using two viscoelastic elements in series to represent molecular friction within the fibril and viscous shear relaxation of the matrix between fibrils respectively (Puxkandl, Zizak et al. 2002). Viscous fibril rearrangement representing the contribution of glycosaminoglycan cross links was implemented in a fibre-reinforced continuum model (Ciarletta, Micera et al. 2006), and later extended to consider the damage of these hypothesised cross links (Ciarletta and Ben Amar 2009). Creep, relaxation and strain stiffening behaviours were captured by a fibre-reinforced Zener model including a recruitment process (Sopakayang and De Vita 2011). Poroelastic constitutive models, explicitly representing solid and one or more liquid phases, have been successfully used to describe this highly hydrated tissue. Implemented as part of a hyperelastic fibre-reinforced continuum poroviscoelasticity was able to describe both quasi-static and dynamic tests, as well as the direction of the fluid flow in rat Achilles tendons (Khayyeri, Gustafsson et al. 2015; Khayyeri, Longo et al. 2016).

As pointed out in the previous chapter, entire tendons may be divided into fascicles. The interfascicular matrix is currently the focus of many works due to its contribution to multiple mechanical functions of tendons (Thorpe, Godinho et al. 2015).

The mechanics of multiple fascicles can be addressed via wrapping algorithms, such as those implemented in musculoskeletal modelling software (Gao, Damsgaard et al. 2002).

The models presented in the section above assume an affine behaviour of the fibrils in the material and provide a good understanding of the tissue-level behaviour of tendons, especially in the central, approximately linear part of the stress–strain curve. However, only few models could also capture the low strain behaviour governed by processes of fibril recruitment and crimp straightening. Therefore we consider explicit models of fibril mechanics in the next section.

3.2.2 Fibril level models

Modelling the mechanical behaviour of fibres and fibrils explicitly is essential for understanding tendon mechanobiology since the heterogeneous arrangements of these structures determine the mechanical signals transduced by cells. Initial discrete modelling efforts focused on the crimped pattern of collagen fibrils in tendon tissue, represented as helices (Beskos and Jenkins 1975), sinusoids (Comninou and Yannas 1976), elastica (Buckley, Lloyd et al. 1980) or with a kinematic chain (Lanir 1979; Kastelic, Palley et al. 1980; Stouffer, Butler et al. 1985; Belkoff and Haut 1992).

A different approach assigned individual fibrils a bilinear stress strain curve which when integrated over the whole tissue fitted the observed non-linearity (Kwan and Woo 1989). This theme is continued with recent use of a helical spring model for fibrils to fit whole tendon stress-stretch data (Grytz and Meschke 2009), acknowledging the limitation of using just one mechanism to account for non-linearity which is known to arise from multiple causes.

Models that include fibril-matrix interaction enable a more sophisticated set of questions to be addressed. Models of the tapered shape (Goh, Meakin et al. 2005) and the

slenderness of fibrils (Goh, Meakin et al. 2007) confirmed the role of stress transfer through the matrix for the tissue level properties of tendon. Helically arranged crimped fibrils embedded in a matrix were able to account for both non-linearity and the high Poisson's ratio of tendon (Reese, Maas et al. 2010). The high Poisson's ratio and fluid exudation under tensile load were addressed by an explicit model of fibrils connected by 'interfibrillar links' embedded in a poroelastic matrix (Ahmadzadeh, Freedman et al. 2015). Together with tensile experiments on enzyme treated tendons, a model with straight fibrils with interconnections representing glycosaminoglycans (GAGs) cast doubt on the theory that these components provide interfibrillar shear load transmission (Fessel and Snedeker 2011). In models of partially-cut tendons a shear lag fibril-matrix load transfer was required to adequately represent the dependence of properties on cut size, and fidelity improved as the number of simulated fibrils increased (Pensalfini, Duenwald-Kuehl et al. 2014).

3.2.3 Cell level models

Tissue microstructure, composition, and the mechanical environment adjacent to cells may be different from that of the 'bulk' extracellular matrix. In cartilage, models have shown that this pericellular matrix has an important role in modulating a chondrocyte's environment (Kim, Guilak et al. 2010). An FEA model of tendon including spatially varying fibril recruitment, poroelastic extracellular and highly compliant pericellular matrix estimated the fluid shear stress around ovoid tendon cells (Lavagnino, Arnoczky et al. 2008).

There is an extensive literature addressing the mechanical modelling of cells. Thus, the reader is referred to an excellent recent review by Rodriguez (Rodriguez, McGarry et al. 2013). For cells that are remodelling tendon under non-rupture conditions, two

important models that could report quantities relevant to the mechanotransduction mechanisms identified in Figure 3.1 are detailed. An elastic model representing a single cell on a 2D surface including pre-stressed actin and tubulin cytoskeletal elements as well as the nucleus and its actin cortex was able to provide good corroboration with atomic force microscopy (AFM) indentation experiments on two cell lines before and after cytoskeletal disruption (Barreto, Clausen et al. 2013). This model was later modified to include the primary cilium of the cell, a well-known mechanosensory organelle (Khayyeri et al 2015). Another elastic model, aimed at understanding a spherical cell seeded into a collagen gel, represented the cytoplasm, cytoskeleton and nuclear envelope (Ujihara, Nakamura et al. 2015). Cytoskeletal elements were randomly oriented and the effects of uniaxial tension, compression and shear applied to the whole gel were investigated. Counterintuitively, macroscopic uniaxial tension increased cytoskeletal compression, while macroscopic compression increased cytoskeletal tension, and shear appeared not to change the loading pattern.

3.3 Adaptive models

The longest-standing application of adaptive mechanobiological models has been directed towards the understanding of bone tissue during development, repair, and remodelling of bone tissue (Carter and Wong 1988; Carter, Beaupre et al. 1998; Lacroix and Prendergast 2002; Isaksson, Wilson et al. 2006).

These iterative approaches use a domain defined in finite element analysis (FEA) to link continuum level mechanical quantities computed from the stress or strain tensors to local tissue change, often represented by change in material properties. This requires the identification of thresholds, defining the ranges of biophysical stimuli for which a

particular tissue type can exist (Perren and Cordey 1980). Excellent reviews of this field are available (Isaksson 2012; Khayyeri, Isaksson et al. 2015).

One approach used a poroelastic finite element model to represent the macroscopic geometry of an animal model for bone healing (Lacroix and Prendergast 2002). Together with thresholds of octahedral shear strain and pore fluid flow, the tissue phenotype of each element and hence its stiffness was updated iteratively (Fig. 3.2). The FEA geometric domain also lends itself to modelling diffusion of growth factors or cells, which can then influence the rules for tissue differentiation (Geris, Andreykiv et al. 2004; Isaksson, van Donkelaar et al. 2008a). These models are well accepted to capture bone tissue mechano-response during healing, and have been widely used to design bone regeneration therapy (Lacroix, Planell et al. 2009; Boccaccio, Uva et al. 2016).

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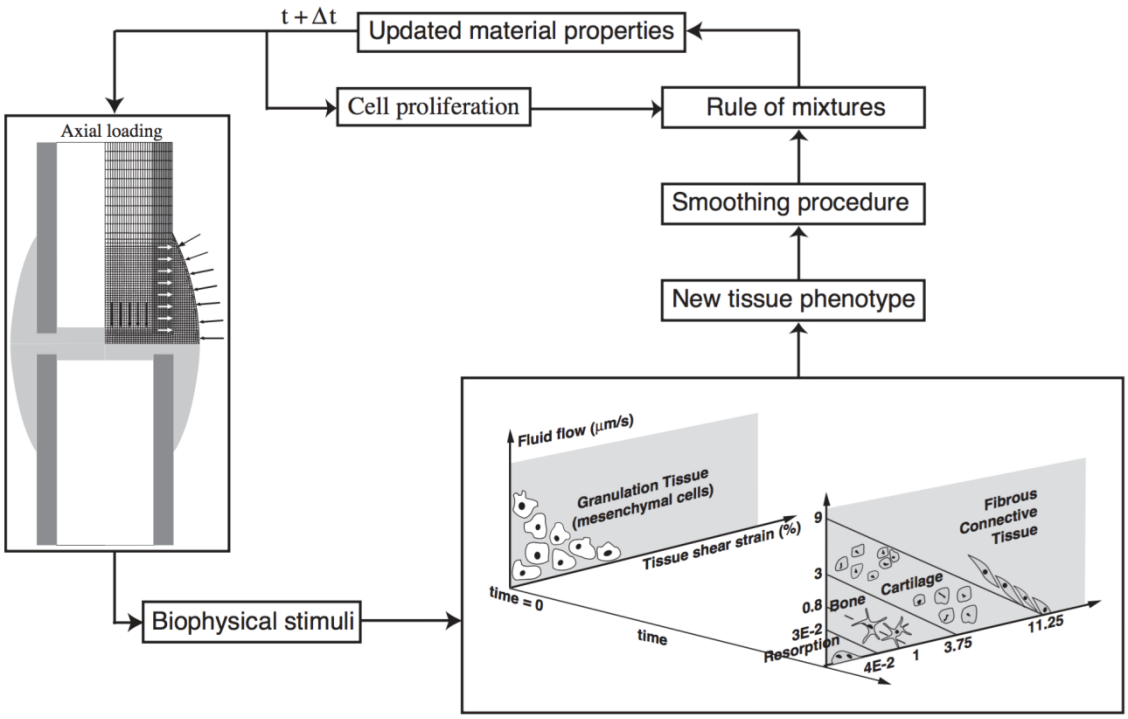

Figure 3.2. Scheme for bone healing adaptive model. With permission (Lacroix and Prendergast 2002)

A different FEA approach was proposed to study the mechanical conditions that produce dysplasia in the prenatal hip joint, linking the increase in element size in a growth region to octahedral shear stress history (Shefelbine and Carter 2004). This concept was extended into using isotropic thermal expansion and remeshing capability to understand the mechanobiological component of joint morphogenesis predicting the role of different movements on formation of hinge and ball and socket joints (Giorgi, Carriero et al. 2014). The same strategy has been used successfully to predict cortical adaptation to externally applied load in mouse tibia (Pereira, Javaheri et al. 2015).

An adaptive continuum mechanics approach implemented using FEA has also been used to model isotropic behaviours of skin expansion for reconstructive surgery (Zollner, Buganza Tepole et al. 2012). This used a growth tensor proportional to local strain within physiological thresholds and was able to predict patient-specific patterns of skin expansion. Other groups have used the combination of finite element models and a growth tensor formulation to successfully capture rapid embryonic epithelial wound healing (Wyczalkowski, Varner et al. 2013). Reaction diffusion equations modelling inflammatory factor and cell migration and collagen deposition in skin wounds using continuum mechanics representations predicted wound shape effects on healing speed (Buganza Tepole and Kuhl 2016).

A different aspect of tissue mechanobiology can be captured by modelling change of fibril orientation using an appropriate constitutive model. Reorientation of collagen fibrils in cartilage due to changing load patterns was represented using FEA and an algorithm rotating the local fibril direction towards an assumed local preferred direction (Wilson, Driessen et al. 2006). This approach was further employed in predicting the development of collagen fibril angular distributions in cardiovascular tissues from an assumed uniform distribution under physiological loading (Driessen, Cox et al. 2008).

Progressive degeneration of collagen in articular cartilage during development of osteoarthritis was also recently described (Mononen, Tanska et al. 2016). In the algorithm, the collagen network stiffness in cartilage was reduced iteratively when a maximum principal stress criteria was exceeded. The developed algorithm was tested and validated against experimental baseline and 4-year follow-up Kellgren-Lawrence grades, indicating different levels of cartilage degeneration at the tibiofemoral contact region (Mononen, Tanska et al. 2016).

Vascular mechanobiological modelling has an added complexity of a considerable physiological pre-stress of the matrix (Cyron and Humphrey 2017). This requires the definition of an attachment or deposition stretch, the stretch of new fibrils synthesised by cells when they are incorporated into the matrix (Humphrey and Rajagopal 2002). This concept was applied in the first microstructurally-based remodelling simulation of an abdominal aortic aneurysm (Watton, Hill et al. 2004). This continuum model used existing constitutive equations for medial and adventitial layers (Holzapfel, Gasser et al. 2000) adapted to incorporate collagen fibril recruitment. Remodelling was represented by coupling the rate of change of either collagen density or recruitment stretch to the difference between current fibril stretch and attachment stretch, and an axisymmetric FEA implementation predicted aneurysm expansion consistent with in vivo and other in silico models.

This concept was sophisticated, introducing triangular distribution functions to describe both recruitment and attachment stretches and further to model the diffusion and reaction of factors important in collagen synthesis and degradation alongside cells to represent aneurysm development and treatment (Aparício, Thompson et al. 2016). This computational model was able to predict the response of an animal aneurysm model to a therapeutic increase in TGF-beta.

A simple analytical mechanobiological model addressed the prediction of tendon cross-sectional area, modulus and tensile strength under a daily strain stimulus during maturation (Wren, Beaupre et al. 1998) (Fig. 3.3). The rates of change were due to the sum of a decaying biological effect (aging) and the difference of the stimulus from a homeostatic value. Comparison with published data on normal, reduced and overloaded tendons showed reasonable agreement. Developments of the model enabled prediction of adaptation following exercise or immobilisation (Wren, Beaupre et al. 2000b). An adaptive poroelastic FEA model of a tendon wrapping around a bone surface using hydrostatic pressure as a stimulus for adaptation predicted decreased permeability at the wrapping surface, suggesting tissue in the region had become more cartilaginous in character (Wren, Beaupre et al. 2000a).

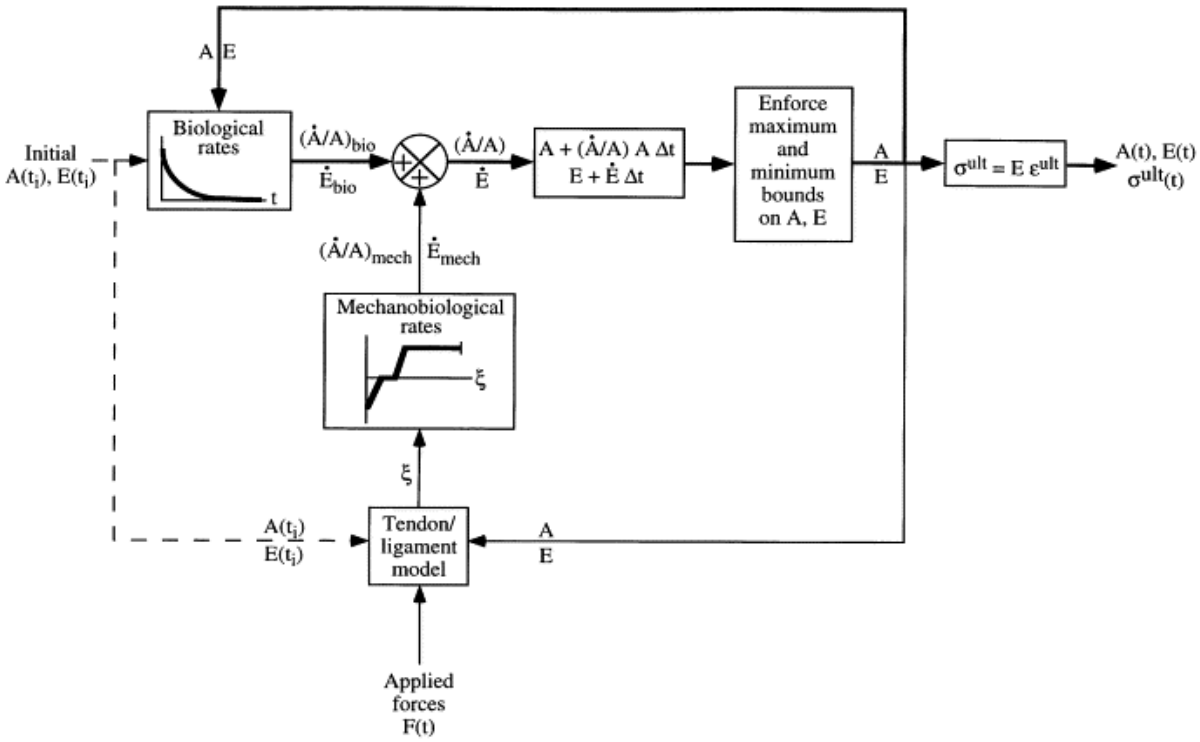

Figure 3.3. Analytical mechanobiological model of tendon / ligament with permission (Wren, Beaupre et al. 1998)

As evidenced above the wide benefits in answering clinically relevant questions that mechanobiological modelling can provide have yet to be realised in tendons. Many different modelling approaches are already available from studies of other tissues, which have huge potential to be further developed to also address specific tendon applications.

3.4 Conclusions

Mechanobiological modelling of tendons has the potential to revolutionise our understanding of tendon adaptation, repair and disease and to point the way to new therapies. Despite encouraging early results, the full range of computational mechanobiological modelling tools has not been applied to study tendons. Biomechanical models using the full range of data now available for the microstructural environment of the tendon cell and adaptive models exploiting techniques developed to represent recruitment, attachment and pre-stress in vascular mechanobiology are two of the lowest hanging fruit.

This approach needs to be developed in parallel with an increased availability of high quality longitudinal data from medical imaging, biochemistry, histological and biomechanical studies either directly in the clinic or using animal models. Such data is lacking for tendon adaptation, healing and degeneration and but is essential to enable validation of computational modelling frameworks to achieve clinical impact. This impact will be of increasing clinical importance to maintain musculoskeletal health in an active, aging population. Tendon can also provide a suite of easily accessible in vitro and in vivo models of a general connective tissue containing fibrillar collagen, elastin and glycosaminoglycans, suitable as a first step in developing protocols and studies for more complex and less easily accessible tissues.

4

A hyperelastic fibre-reinforced continuum model of healing tendons with distributed collagen fibre

This chapter describes the work performed to develop a microstructurally inspired constitutive model, which is able to characterise the biomechanical behaviours of healing tendons at different timepoints. It includes a brief overview of models used by previous works for simulating soft tissue mechanics, and is followed by a suggestion of a model based on continuum fibre-reinforced formulation to be used in this study. A scheme for model fitting to experimental data is outlined thereafter, followed by finite element model development. Finally, a design of experiment method used for parametric studies is described, identifying which parameters are influential in capturing healing tendons' tensile behaviour.

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4.1 Introduction

Previous chapters have emphasised the urgent need to further explore mechanobiological behaviours of healing tendons in a quest to develop more efficient treatments of ruptured tendons. Computational approaches have received much attention in further elucidating the underlying the mechanobiological mechanisms of other tissues, however few studies have addressed tendons (see e.g. Chapter 3). The work presented in this chapter is therefore carried out, as preliminary work in the computational simulation of healing tendons. The importance of collagen fibre alignment to this process as discussed in Chapter 2, has motivated the novel application of a microstructurally inspired constitutive model.

The disabling condition of ruptured tendons, which is primarily associated with substantial pain, may take many months, or even years of healing until full function has returned. Numerous studies, both in animal models and in patients, have shown that mechanical loading has a significant impact on the speed and efficiency of healing (Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Killian, Cavinatto et al. 2012; Wang, Guo et al. 2012) as discussed in detail in Chapter 2, section 2.2.4. However, the optimal loading regime remains unclear and the detailed mechanobiological mechanisms involved are not fully understood.

Computational approaches have been widely used in mechanobiological modelling of bone healing to enable predictions of tissue differentiation and improve the understanding of both the mechanical and biological mechanisms at play (Isaksson 2012). In order to apply this approach to tendon healing, a continuum constitutive model that can both represent the changing tendon mechanics during healing and also represent the proposed biophysical stimuli for the cells involved is required.

As discussed in Chapter 3, section 3.2.1, continuum constitutive models developed for the quasi-static properties of the whole tendons may be classed as either phenomenological, or microstructural (Weiss and Gardiner 2001). Phenomenological models with parameters that do not provide a direct physical interpretation have successfully been used to model tendon macroscopic behaviour (Woo 1982; Woo 1986; Woo, Johnson et al. 1993). However, microstructural models are required to capture the complex changes in density, organisation and alignment of collagen fibres during healing. With the representation of multi-scale deformation mechanisms the complexity of such models rapidly increases, some requiring as many as seven independent parameters to be fitted (Hurschler, Loitz-Ramage et al. 1997; Reese, Maas et al. 2010). A compromise approach is to use some microstructural parameters that can be directly measured together with phenomenological parameters enabling a simple yet meaningful model to be analysed. Scanning electron microscopy studies in rat and rabbit models of tendon healing show how the callus develops from an isotropic gel shortly after rupture to a highly aligned, anisotropic material when healed (Fig. 4.1) (Sasaki, Yamamoto et al. 2012, Enwemeka 1989). This motivates the choice of a fibre-reinforced continuum constitutive model.

One such model with distributed collagen orientations introduced by Gasser-Ogden-Holzapfel (GOH) has been widely used to simulate soft tissue mechanics (Holzapfel, Gasser et al. 2000; Gasser, Ogden et al. 2006). As well as being used extensively to model arterial walls, this model effectively captured the link between variations in collagen arrangement and dispersion, and the tensile behaviour of the tissue in the skin dermis (Ní Annaidh, Bruyère et al. 2012). It has also been used successfully to model wound healing alongside transport models for cellular and chemical species

(Valero, Javierre et al. 2015). To date, however, the GOH model has not been used to simulate the mechanics of healing tendons.

This study aimed to test the ability of the GOH model to capture the elastic behaviour of healing tendons using both curve fitting and a design of experiments (DOE) parametric study to examine the parameter sensitivity. Tensile test data from a rat model of Achilles tendon healing were used throughout the study (Eliasson, Andersson et al. 2009).

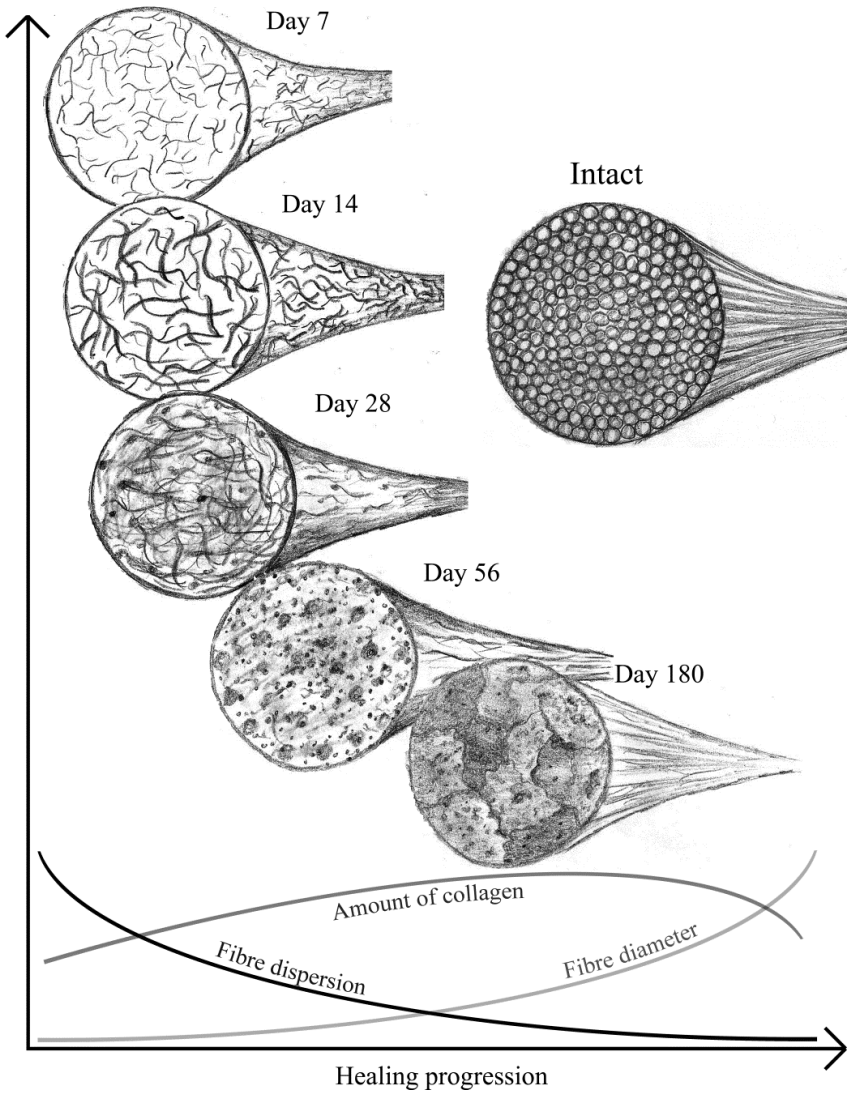


Figure 4.1. Schematic structure of fibre dispersion, diameter and amount in healing tendons. Graphs illustrate patterns of changes during the healing process

4.2 Materials and methods

4.2.1 Gasser-Ogden-Holzapfel model

This section explains how the GOH model could be adapted to model the tensile behaviours of healing tendons by assuming that the tissues were incompressible materials with two preferred direction of fibres, aligned along the unit vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ (Fig. 4.2). The theoretical development provided below is taken directly from Ni Annaidh (Ní Annaidh, Bruyère et al. 2012) who utilised this model to simulate the hyperelasticity of skin.

The strain energy density, $\boldsymbol{\Psi}$, of the GOH model has the form of

$$\boldsymbol{\Psi} = \boldsymbol{\Psi}\,(\mathbf{C}, \mathbf{H}_1, \mathbf{H}_2) \tag{1}$$

where $\mathbf{C}$ denotes the right Cauchy-Green strain tensor and the structure tensors $\mathbf{H}_1$, $\mathbf{H}_2$. The structure tensors depend on unit vectors $\mathbf{a}_1$ and $\mathbf{a}_2$ and on the dispersion factors κ_1, κ_2, respectively, as shown below:

$$\mathbf{H}_i = \kappa_i \mathbf{I} + (1 - 3\,\kappa_i)\,\mathbf{a}_1 \otimes \mathbf{a}_2, \qquad (i = 1, 2) \tag{2}$$

and that $\boldsymbol{\Psi}$ depends on I_1 = tr($\mathbf{C}$), tr($\mathbf{H}_1\mathbf{C}$), and tr($\mathbf{H}_2\mathbf{C}$), as shown below:

$$\boldsymbol{\Psi} = \frac{\mu}{2}(I_1 - 3) + \mu \sum_{i=1}^{2} \frac{k_{i1}}{2k_{i2}} \left\{ e^{k_{i2}\,[\mathrm{tr}(\mathbf{H}_i\,\mathbf{C}) - 1]^2} - 1 \right\} \tag{3}$$

where μ, k_{i1}, k_{i2} are positive material constants, and from Eq. (2):

$$\mathrm{tr}(\mathbf{H}_i\mathbf{C}) = \kappa_i \boldsymbol{I}_i + (1 - 3\kappa_i)\, \boldsymbol{I}_{4i}, \qquad \textbf{(4)}$$

with $\boldsymbol{I}_{4i} \equiv \mathbf{a}_i \cdot \mathbf{C}\mathbf{a}_i$, two anisotropic invariants. Note that the constitutive parameter μ has the dimensions of stress: it would be the shear modulus of the solid if there were no fibres ($k_{i1} = 0$); whilst the parameter k_{i1} is a stress-like parameter and k_{i2} is a dimensionless parameter to be determined from mechanical tests of the tissue.

A model of uniaxial tensile test of healing tendons can be achieved when two families of fibres are mechanically equivalent $k_{11} = k_{21} \equiv k_1$ (say) and $k_{12} = k_{22} \equiv k_2$ (say), with the same dispersion factor $\kappa_1 = \kappa_2 \equiv \kappa$ (say), and when the tension occurs along the bisector of $\mathbf{a}_1$ and $\mathbf{a}_2$ (Fig. 4.2). Let γ be the angle between $\mathbf{a}_1$ and the tensile direction, therefore:

$$\mathbf{a}_1 = \cos\gamma\mathbf{i} + \sin\gamma\mathbf{j}, \qquad \mathbf{a}_2 = \cos\gamma\mathbf{i} - \sin\gamma\mathbf{j} \qquad \textbf{(5)}$$

where **i** is the unit vector in the direction of tension, and **j** is the unit vector in the lateral direction. The stretch ratios along those unit vectors are λ_1 and λ_2, respectively. Then, $I_{41} = I_{42} = \lambda_1^2\cos^2\gamma + \lambda_2^2\sin^2\gamma \equiv I_4$ (say), and $\boldsymbol{\Psi}$ reduces to:

$$\boldsymbol{\Psi} = \frac{\mu}{2}(I_1 - 3) + \mu\frac{k_1}{k_2}\left\{e^{k_2[\kappa I_1 + (1-3\kappa)I_4 - 1]^2} - 1\right\} \qquad \textbf{(6)}$$

resulting in the following expression for $\boldsymbol{\sigma}$, the Cauchy stress tensor:

$$\boldsymbol{\sigma} = -p\mathbf{I} + 2\frac{\partial \Psi}{\partial I_1}\mathbf{F}\mathbf{F}^T + \frac{\partial \Psi}{\partial I_4}[\mathbf{Fa_1} \otimes \mathbf{Fa_1} + \mathbf{Fa_2} \otimes \mathbf{Fa_2}], \quad \textbf{(7)}$$

Here, p is a Lagrange multiplier introduced by the internal constraint of incompressibility, while **F** is the deformation gradient. Note that $\mathbf{Fa_1} \otimes \mathbf{Fa_1} + \mathbf{Fa_2} \otimes \mathbf{Fa_2} = 2(\lambda_1 \cos\gamma)^2\mathbf{i} \otimes \mathbf{i} + 2(\lambda_2 \sin\gamma)^2\,\mathbf{j} \otimes \mathbf{j}$, showing that $\boldsymbol{\sigma}$ is diagonal in the { **i, j, k** } basis. Its components are

$$\begin{aligned} \sigma_{11} &= -p + 2(\boldsymbol{\Psi}_1 + \boldsymbol{\Psi_4}\cos^2\gamma)\lambda_1^2 \neq 0, \\ \sigma_{22} &= -p + 2(\boldsymbol{\Psi}_1 + \boldsymbol{\Psi_4}\sin^2\gamma)\lambda_2^2 = 0, \\ \sigma_{33} &= -p + 2\boldsymbol{\Psi}_1\lambda_1^{-2}\lambda_2^{-2} = 0, \end{aligned} \quad \textbf{(8)}$$

where

$$\begin{aligned} 2\boldsymbol{\Psi}_1 &= \mu(1 + 4k_1\kappa\alpha e^{k_2\alpha^2}), \\ 2\boldsymbol{\Psi}_4 &= 4\mu k_1(1 - 3\kappa)\alpha e^{k_2\alpha^2}), \\ \alpha &= \kappa(\lambda_1^2 + \lambda_2^2 + \lambda_1^{-2}\lambda_2^{-2}) + (1 - 3\kappa)(\lambda_1^2\cos^2\gamma + \lambda_2^2\sin^2\gamma) - 1 \end{aligned} \quad \textbf{(9)}$$

p is then eliminated from the stress components to obtain the equations below:

$$\sigma_{11} = \mu(\lambda_1^2 - \lambda_1^{-2}\lambda_2^{-2}) + 4\mu k_1\alpha e^{k_2\alpha^2}[\kappa(\lambda_1^2 - \lambda_1^{-2}\lambda_2^{-2}) + (1 - 3\kappa)\lambda_1^2\cos^2\gamma] \quad \textbf{(10)}$$

$$0 = \lambda_2^2 - \lambda_1^{-2}\lambda_2^{-2} + 4k_1\alpha e^{k_2\alpha^2}[\kappa(\lambda_2^2 - \lambda_1^{-2}\lambda_2^{-2}) + (1 - 3\kappa)\lambda_2^2\sin^2\gamma] \quad \textbf{(11)}$$

Equation (11) describes the relationship between the tensile stretch and the lateral stretch and allows, implicitly, λ_2 to be expressed in terms of λ_1. The σ_{11}–

λ_1 , stress-stretch relationship can be obtained by substituting (11) into (10). When linearised in the neighbourhood of small strains, $\lambda_i \cong 1 + \varepsilon_i$, these equations have the following forms:

$$\sigma_{11} = 4\mu\,[1 + 2k_1(1 - 3\kappa)^2\cos^4\gamma]\varepsilon_1 + 2\mu[1 + 4k_1(1 - 3\kappa)^2\sin^2\gamma\cos^2\gamma]\varepsilon_2, \quad \textbf{(12)}$$

$$0 = [1 + 4k_1(1 - 3\kappa)^2\cos^2\gamma\sin^2\gamma]\varepsilon_1 + 2[1 + 2k_1(1 - 3\kappa)^2\sin^4\gamma]\varepsilon_2 \quad \textbf{(13)}$$

This demonstrates that the constitutive parameters μ and k_1 are related to the early stages of the tensile tests, whilst k_2 is the stiffening parameter, related to the latter (non-linear) stages of the tensile tests. Looking for the relationship between σ_{11} and ε_1, by solving (13) for ε_2; and substitution into (12), the linear stress-strain relation $\sigma_{11} = E_1\varepsilon_1$ was obtained, where E_1 is the infinitesimal Young's modulus in the 1-direction, found here as:

$$E_1 = \frac{3+8k_1(1-3\kappa)^2(1-3\cos^2\gamma\sin^2\gamma)}{1+2k_1(1-3\kappa)^2\sin^4\gamma}\mu \quad \textbf{(14)}$$

Hence, by plotting the values of σ_{11} for the linear part of the test, E_1 was determined by linear regression analysis.

The μ value was then determined using equation (14). The remaining material parameters k_1 and k_2 were determined using the nonlinear least squares fitting with experimental test data of Eq. (10), subject to the definition of λ_2 in terms of λ_1 given by Eq. (11). The data fitting was performed using the *lsqnonlin* in MATLAB with the objective function, *Err(k)*, given as:

$$Err(k) = \sum_{i=1}^{n} (y_i^{\exp} - y_i^{\mathrm{model}(k)})^2 \quad \textbf{(15)}$$

where n is the number of experimental data points, $y_i^{\exp}$ is the experimentally measured value of the stress of a tensile tested sample (Eliasson, Andersson et al. 2009), and $y_i^{\mathrm{model}(k)}$ is the stress predicted by the model using the current set of material parameters, k, which are k_1, k_2, and μ. The μ in the anisotropic term of Eq. (6) is not a standard for the GOH model, and was included here for ease of calculation. The conventions used in this study are related to the GOH model via $c_{10} = \mu/2$, $k_1' = k_1 \mu$ and $k_2' = k_2$, where c_{10} represents a combination of the non-collageneous matrix neo-Hookean shear modulus and its volume fraction, while k_1' and k_2' represent a combination of the collagen stiffness and collagen volume fraction (Fig. 4.2).

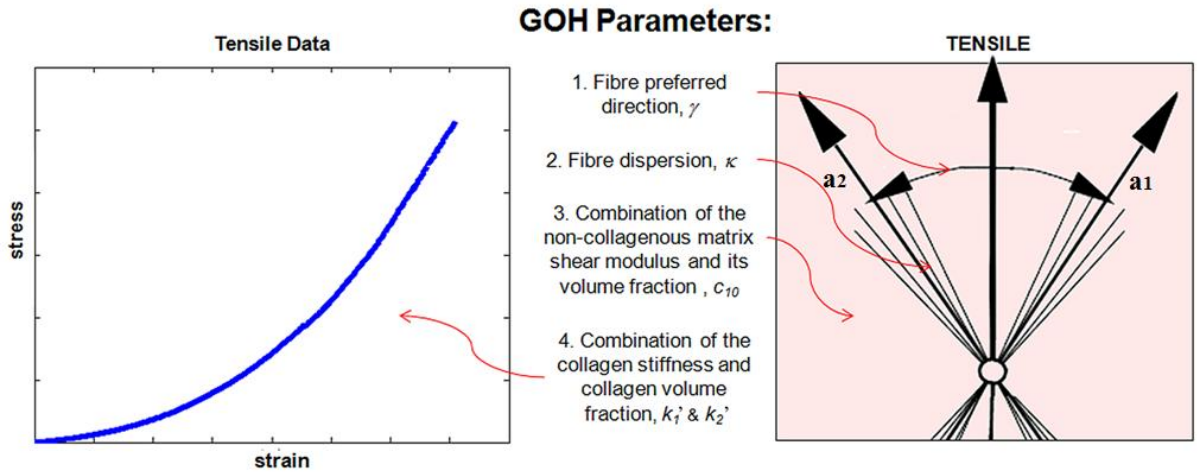


Figure 4.2. Parameters of the GOH model. Please note that γ was set to zero

This scheme was used to fit uniaxial tensile force-displacement data from a rat Achilles tendon healing model characterising the callus at 3, 8, 14 and 21 days after transection and in intact tendons (N = 4 - 5 in each group) (Eliasson, Andersson et al.

2009). The fit was performed to data with force greater than 0.5 N up to the point of rupture, which was determined at the maximum gradient.

The coefficient of determination, R^2 was used to gauge the goodness of fit. To ensure that the function was fitted at a global rather than local minima, a large range of initial parameter values was manually tested, i.e. plus minus 200% with 10% step size. We hypothesised that the fitted values are of global minima if they were strictly independent of initial values.

4.2.2 Selection of structural parameters

The structural parameters were defined based on literature. Collagen fibres are primarily found axially aligned throughout tendons (Wang 2006), so the alignment angle γ was set to 0^o. Following scanning electron microscopy studies of healing tendons (Sasaki, Yamamoto et al. 2012) we assumed that the alignment dispersion, κ, should decrease during healing. We defined the absolute values within the range of 0.33 to 0.00 for κ set by Gasser (Gasser, Ogden et al. 2006), using three criteria based on the assumed direction of change of the constitutive parameters during healing. The fibre stiffness parameters, k_1' and k_2' should increase over healing, as both more collagen is formed and the collagen crosslinks increase during repair (Fessel, Gerber et al. 2012). The neo-Hookean shear modulus of the non-collagenous matrix, c_{10}, will reduce as the matrix volume fraction reduces during healing (Guo, Peng et al. 2007). Based on these premises, comparative analyses were performed between different ranges of κ. We found that values of 0.25, 0.24, 0.23, 0.22, 0.00 for samples of day 3, 8, 14, 21, intact, respectively, produced values that generally meet the criteria above. These values were used throughout the study.

4.2.3 Finite element representation

The ability of the GOH model to simulate tendons undergoing repair was further tested in an axisymmetric finite element (FE) environment, assuming a perfect cylindrical shape. Five sample-specific axisymmetric models, one per timepoint (day 3, 8, 14, 21 post-rupture and intact), were created. To choose one (out of five) tendon that was representative for each time point of healing, the sample with the median R^2 value from fitting of the 1D data was selected.

Table 4.1. Parameter values and input used in the finite element analysis. The values of the GOH parameters were obtained from an optimisation procedure whilst data for model dimensions and loading, right before microfailure during a tensile test were taken from Eliassson (Eliasson, Andersson et al. 2009)

Sample	κ	k_1'	k_2'	c_{10}	**Loading Force (N)**	**Dimension (mm)**	
						Length	**Radius**
Day 3	0.25	1.11	5.80	0.167	3.3	6.0	1.2
Day 8	0.24	1.92	13.86	0.081	14.1	8.5	1.6
Day 14	0.23	2.27	7.08	0.179	36.5	8.4	1.8
Day 21	0.22	1.24	3.87	0.107	31.0	7.0	1.9
Intact	0.00	9.13	1.60	4.60e-4	29.9	3.5	0.6

The dimensions and the optimised values of the GOH parameters for each model are shown in Table 4.1. The conventions used by ABAQUS is related to this study via $C_{10} = \mu/2$, $k'_1 = k_1 \mu$ and $k'_2 = k_2$. Please note that the lengths of the models were half of the original tissues as FE analyses were performed using symmetry boundary conditions. Reduced integration hybrid rectangular axisymmetric (CAX8RH) elements were used to

mesh all models. The boundary conditions from the experimental tensile test were mimicked by applying force to the nodes on the top edge, with translation allowed only in the *y* direction, and all rotational degrees of freedom of the nodes were constrained (Fig. 4.3). The other two edges – xsymm and ysymm – which indicate the symmetrical line of the model, were constrained in each particular direction. The model was run as a static analysis in ABAQUS/Standard (v6.12-4, Dassault Systèmes, France) with non-linear geometry, using the NLGEOM to account for large deformations of the tendon. The values of engineering strain were compared with the experimental data and the corresponding R^2 value was calculated.

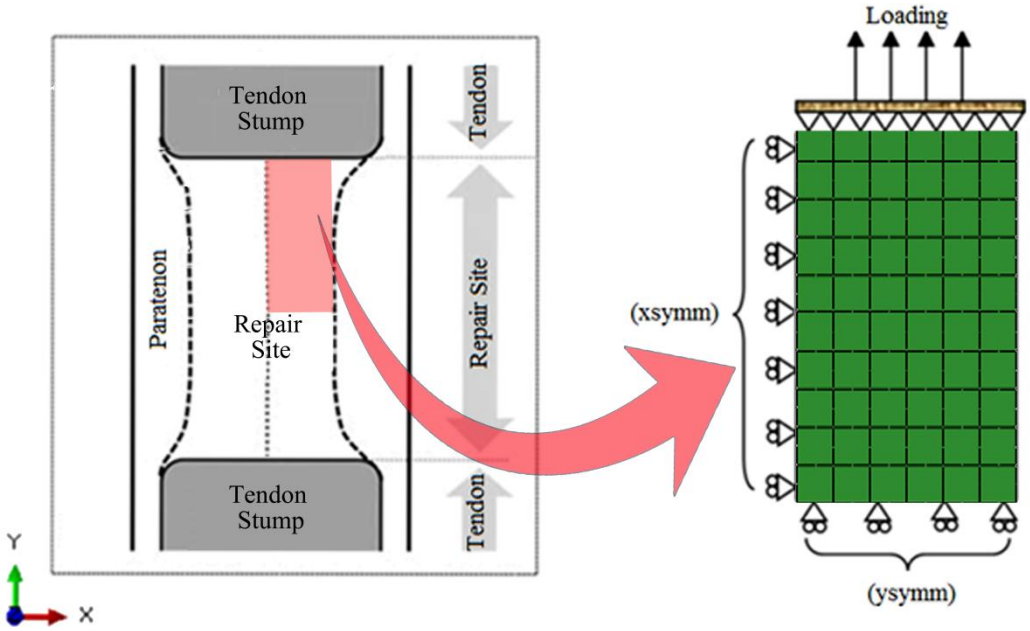


Figure 4.3. Dimensions of the simulated tendon (axisymmetric). xsymm and ysymm indicate that these edges are the symmetrical line of the body with respect to its corresponding axis, and are constrained in that particular direction. For instance, the xsymm edge was constrained in the x direction, which only allows the body to move in the y direction.

4.2.4 Parametric study

A Design of Experiments approach based on fractional factorial designs was used to investigate the importance of the GOH parameters for characterising the mechanical behaviour of healing Achilles tendons (Isaksson, van Donkelaar et al. 2008b; Isaksson, van Donkelaar et al. 2009). Fractional factorial designs are experimental designs that consist of carefully chosen subsets or fractions of the experimental runs needed to complete full factorial designs (Phadke 1995; Montgomery 2006). Hence, they present an efficient method to study the effects of two or more factors on a specific process. For this study a three-level central composite design (CCD) was chosen. CCD is a type of fractional factorial design that uses a second order model for the response surface without needing to use a complete three-level factorial design (Funkenbusch 2005; Isaksson, van Donkelaar et al. 2009). The four GOH parameters were examined – k_1', k_2', c_{10} and κ – and are referred to as control factors, while the values they take are called levels. Each combination of control factor levels that is evaluated is called a treatment condition (Phadke 1995; Isaksson, van Donkelaar et al. 2008b). The chosen parameter space was based on the variances of the optimised values. The low (-1) and high (+1) levels at each timepoint were set according to the variation in the experimental data as two standard deviations from the mean value. The mean value was taken as the normal (0) (Table 4.2). The experiment included a total of 31 treatment conditions at every timepoint of healing.

Table 4.2. The parameter space for the DOE

Group	**Levels**		
	-1	**0**	**1**
	k_1'		
day3	0.6125	1.3599	2.1073
day8	0.9270	2.0059	3.0847

day14	0.0001	2.8760	6.3391
day21	0.3247	1.4412	2.5577
Intact	4.2104	11.5907	18.9710
	k_2'		
day3	0.0001*	8.6435	25.8065
day8	0.0001*	8.0924	16.8297
day14	2.9620	5.8215	8.6809
day21	1.1823	4.8871	8.5918
Intact	0.0001*	3.2600	8.7982
	c_{10}		
day3	0.0898	0.2007	0.3115
day8	0.0269	0.1026	0.1783
day14	0.1141	0.1773	0.2405
day21	0.0475	0.1057	0.1638
Intact	2.11e-4	5.83e-4	9.55e-4
	κ		
day3	0.22	0.25	0.28
day8	0.21	0.24	0.27
day14	0.20	0.23	0.26
day21	0.19	0.22	0.25
Intact**	0.00	0.03	0.06

* this number was chosen as values of the parameters must always be positive

** the assumed normal (level 0) value for the κ for intact sample is 0.00 not 0.03. As its values must always be positive, we chose 0.00 to be the low boundary instead, assuring a broadened positive parameter space

To assess the results obtained from the parametric study, criteria that characterise the performance of the system for each treatment condition were determined. A match target of 1 was set for the coefficient of determination, R^2. Analysis of variance (ANOVA) was used to calculate the normalised percentage of the total sum of squares for each factor, %TSS to measure the contribution of each factor at specific timepoints of healing (Dar, Meakin et al. 2002; Isaksson, van Donkelaar et al. 2009).

4.3 Results

4.3.1 Optimized constitutive parameters

Using the parameter ranges described, it was possible to obtain excellent fits for the GOH model for all samples at all timepoints ($0.9903 < R^2 < 0.9986$) (Fig. 4.4). The means of the optimised values for the constitutive parameters, k_1' and c_{10} show the expected trends over the healing period, with the values of k_1' generally increasing whilst c_{10} is decreasing (Table 4.3). A small drop in k_1' at day 21 was observed. k_2' appears to reduce over healing, contrary to expectations. The standard deviations of each parameter show the level of variability, with the average coefficients of variation, CV% of 37%, 60% and 28% for k_1', k_2' and c_{10}, respectively.

Table 4.3. The optimised model parameters for each group. The mean (of the samples at each group), the standard deviation (SD) and the coefficient of variation (CV) for each parameter and group are calculated

Group	k_1'			k_2'			c_{10}		
	Mean	**SD**	**CV (%)**	**Mean**	**SD**	**CV (%)**	**Mean**	**SD**	**CV (%)**
Day 3	1.36	0.37	27.5	8.64	8.58	99.3	0.20	0.06	27.6
Day 8	2.01	0.54	26.9	8.09	4.37	54.0	0.10	0.04	36.9
Day 14	2.88	1.73	60.2	5.82	1.43	24.6	0.18	0.03	17.8
Day 21	1.44	0.56	38.7	4.89	1.85	37.9	0.11	0.03	27.5
Intact	11.59	3.69	31.8	3.26	2.77	84.9	5.83e-4	1.86e-4	31.9

.

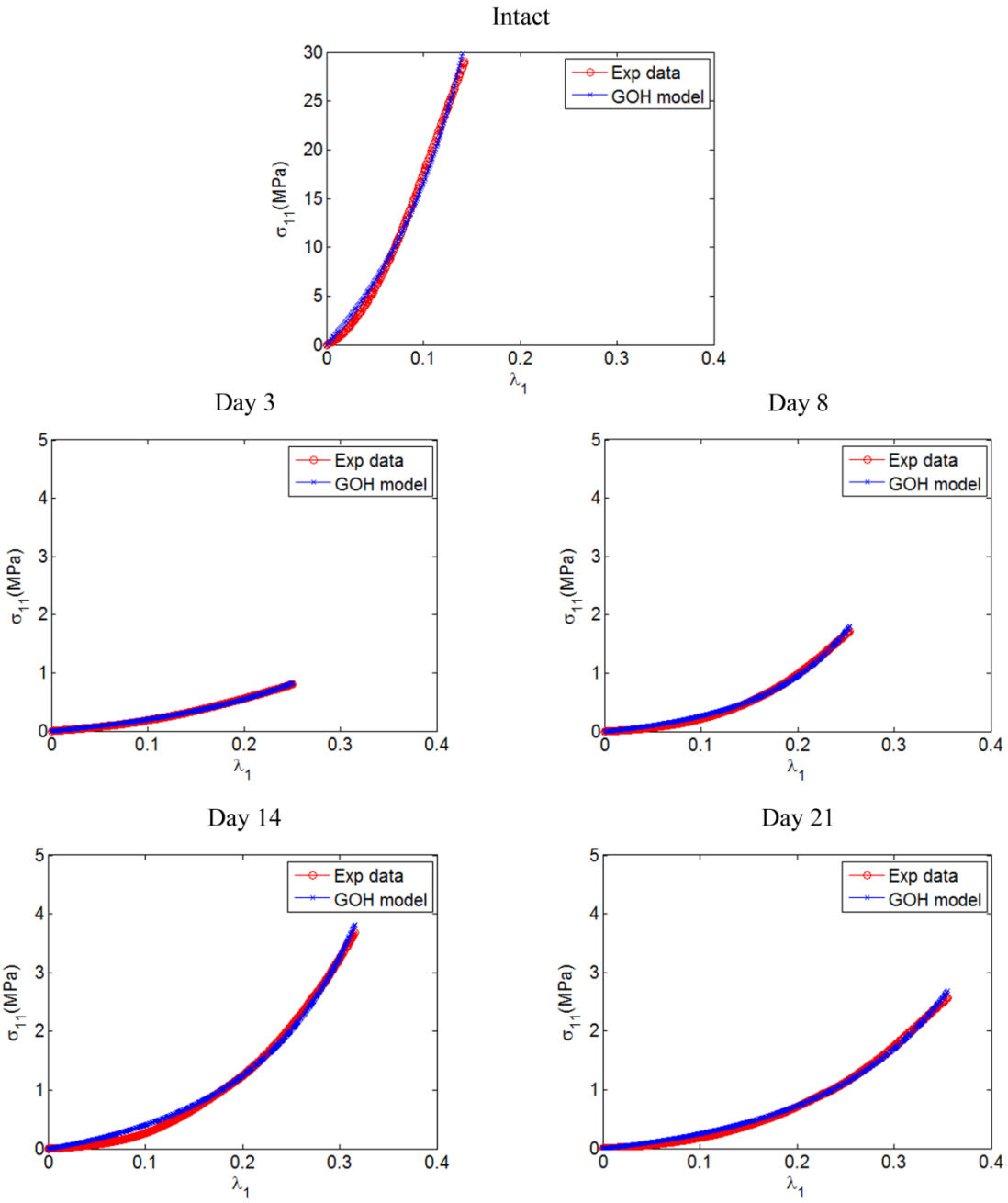


Figure 4.4. Stress-stretch plots of the optimisation procedure of the GOH model against experimental data. Data fitting was based on the sample with the median R^2 value at each specific timepoints over healing, as representative of other samples

4.3.2 Finite element simulations

The finite element studies employing the GOH constitutive model with parameters determined from the optimised values through data fitting showed that the fits remain good ($0.9924 < R^2 < 0.9964$) (Fig. 4.5).

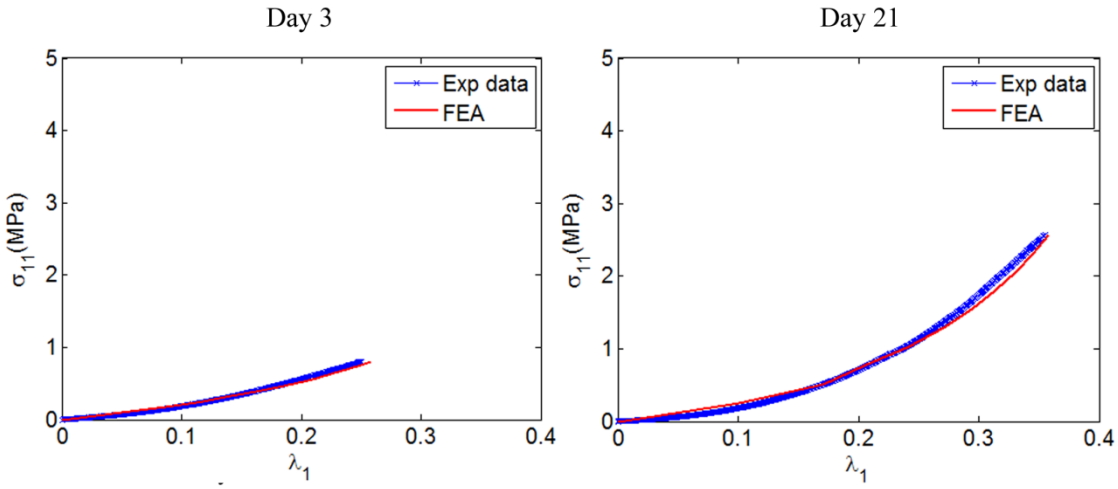


Figure 4.5. Comparison between predicted model response using finite element analyses and experimental tensile data of a healing tendon at day 3 and day 21. The R^2 values were 0.9964 and 0.9959, respectively

4. 3.3 Parametric study

All parameters related to the collagen fibres were found to be highly influential; the parameters describing the dispersion, stiffness at small strain and stiffening behaviour at large strain of the collagen fibres, κ, k_1', and k_2', respectively (Fig. 4.6). The parameter associated with the non-collagenous matrix, c_{10} was found to be the least influential. The importance of two parameters varied during the healing: the importance of the fibre dispersion, κ, was higher during early part of healing and decreased towards the end of the healing and in the intact sample. On the contrary, the small strain fibre stiffness, k_1', increased its influence gradually over healing and was more important in the intact sample.

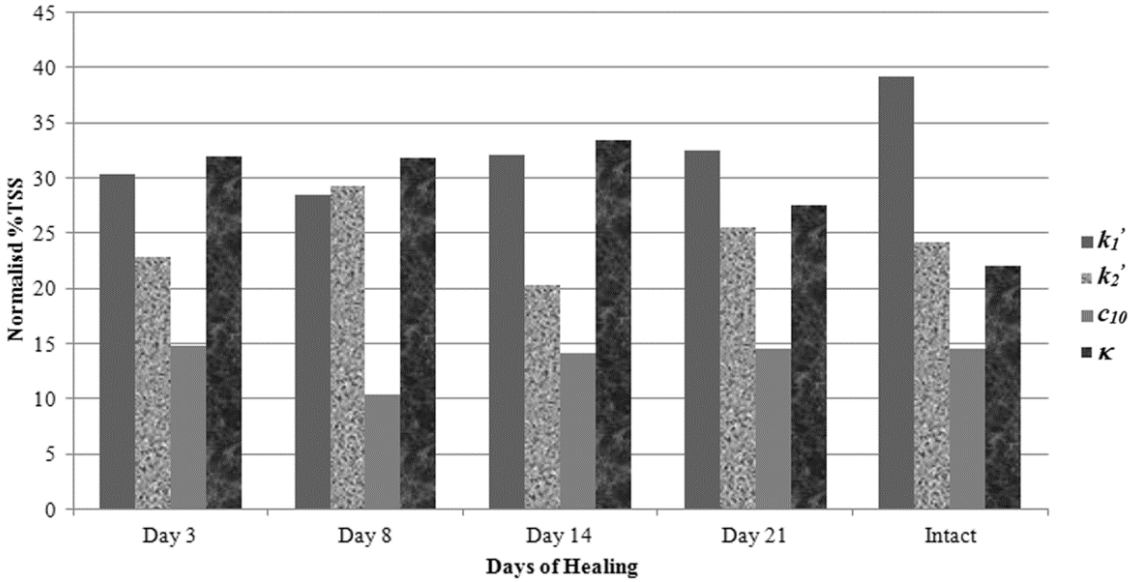


Figure 4.6. ANOVA of each of the outcome variables based on the normalised %TSS

4.4. Discussion

To the best of our knowledge, no previous studies have used the GOH constitutive model to simulate the mechanical behaviour of tendons at various stages of healing, following an extensive literature review carried out in Chapter 3. In this chapter, we have shown that good fits ($R^2 > 0.99$) could be obtained against experimental data from intact tendons and tendons at four different timepoints of healing. Our parametric study showed that the relative importance of the collagen fibre-related GOH parameters somewhat varied during healing, but always remained well above the importance of the non-collagenous matrix parameter.

This study proposes the use of the dispersion of the collagen fibre angular distribution in the GOH model, κ, to characterise the tissue maturation during tendon healing. The reduction in collagen fibre dispersion during healing is evidenced qualitatively by electron microscopy of tendon healing in a rat model (Sasaki, Yamamoto et al. 2012). Previously the GOH model was used together with a quantitative method to measure fibre dispersion to fit the anisotropic properties of skin dermis (Ní Annaidh,

Bruyère et al. 2012). Measured fibre dispersions ranged from 0.0924 to 0.1698, which is somewhat lower than the maximum limit of 0.33. Recently, the GOH model, with a fixed fibre dispersion of 0.1404, was used to model wound healing (Valero, Javierre et al. 2015). However, since there are still no quantitative measurements of collagen dispersion through tendon healing, the dispersion values in the present study were chosen to vary from 0.25 to 0.22 to simulate the reduction of the fibre dispersion in the early stage of healing tendons. This range may seem a little narrow, but it has been analysed ensuring that the resulting optimised values follow the reported literature findings. On that note, we have confirmed that this setting produced k_1' values that generally increased with healing progression. However, in agreement with a drop in reported modulus of elasticity at day 21 (Eliasson, Andersson et al. 2009), k_1' decreased at this timepoint compared with day 14. Eliasson *et al.* speculate that this decreased stiffness might be associated with upregulated tendon-specific genes also observed at this timepoint. On a general note, while the structural parameters in the GOH model may be directly measured in experiment, the phenomenological parameters represent a combination of volume fraction and material property that cannot be measured directly. The changes in the estimated values of these parameters over time therefore reflect changes of both material property and tissue composition. Further information on mechanical and material properties as well as structural change during healing has been discussed in Chapter 2, section 2.2.3.

It is well documented that the elastic modulus of healing tendons increases over time (Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Schepull and Aspenberg 2013). The present study obtained excellent fits of the GOH model at each timepoint, with the collagen fibre stiffness, k_1' increasing during healing. The neo-Hookean shear modulus of the non-collagenous matrix, c_{10} reduced as healing progressed, as expected. These phenomenological parameters show expected trends over time but do not change

monotonically. However, k_2' showed unexpected decreases. These are phenomenological parameters, and their non-monotonic and unexpected changes are likely due to the combined influence of changes in material property, callus volume and composition.

Most of the previous continuum models of tendon were developed by assuming the tissue is similar to other soft tissues e.g. ligament and arteries, especially when describing its time and rate independent elastic behaviour (Weiss and Gardiner 2001) (see Chapter 3 section 3.2.1). An exponential stress-strain relationship based on uniaxial experiments was used by Fung (Fung 1967) to construct a phenomenological model of rabbit mesentery. This model has been widely used in capturing other soft tissues' macroscopic mechanical behaviour (Demiray 1972) e.g. arteries (Chuong and Fung 1983), cartilage (Zopf, Flanagan et al. 2015). However, it lacks structural information and the model parameters are not determined via experimental data (Holzapfel, Ogden et al. 2004).

Previous models to simulate microscale behaviours of tendons have been focused on collagen fibrils and their crimp pattern (see Chapter 3, section 3.2.2). Micromechanical models of helical superstructures successfully predict non-linear behaviour and large Poisson's ratios in ligaments and tendons (Reese, Maas et al. 2010; Shearer 2015). Various other approaches including shear-lag models (Szczesny and Elliott 2014) have been implemented successfully; however, the experimental information required for their use in healing tendon is lacking. Furthermore, this form of microstructural approach is difficult to implement in a finite element analysis, as it requires detailed geometrical representations (e.g. length, diameter, volume) of each fibre with highly refined meshes, along with complex contact modelling between the fibre and its surrounding matrices, which could result in overly complex computations (Xia, Okabe et al. 2002).

Many modelling endeavours have shown the relevance of multi-structural fibre-reinforced constitutive models for understanding the relationship between constituents and

the overall mechanical behaviour of tissues. The simplest such model is an isotropic solid matrix containing one family of fibres, and that has been used successfully to simulate the behaviour of fascia lata and tendons (Weiss, Maker et al. 1996; Natali, Pavan et al. 2005). Another study used two distinct families of fibres to model the interwoven collagen of cruciate ligaments (Hirokawa and Tsuruno 2000). This approach was used by Holzapfel (Holzapfel, Eberlein et al. 1996; Holzapfel and Gasser 2001) in their early model development for the arterial wall. They later introduced a scalar to represent symmetric fibre dispersion, κ (Gasser, Ogden et al. 2006) allowing both the alignment and the organisation of fibres in the tissue to be represented. The very recent non-symmetrical dispersion model (Holzapfel, Niestrawska et al. 2015) may provide an improvement over the symmetric version used here, provided that sufficient histological information is available.

This is the first study to investigate the importance of parameters in a constitutive model of healing tendons using the design of experiment (DOE) method. DOE has been used previously in mechanobiological studies of bone healing (Isaksson, van Donkelaar et al. 2008b; Isaksson, van Donkelaar et al. 2009). Our findings showed that the GOH parameters relating to collagen fibres are the most influential in characterising the tensile behaviour of healing tendons, compared to those associated with the non-collageneous matrix. This agrees with a picture of tendon healing in which the major mechanical changes over time are defined by the initial production of disorganised collagen III, and its gradual remodelling and replacement by organised collagen I. It is also noteworthy that the outcome of the parametric study reflects the experimental setup that was used. In our case, data from static tensile tests was used, which supported the hypothesis that collagen fibres are the main load-bearing components in tendons. A study by Khayyeri (Khayyeri et al. 2015) has shown that the contributions of tendons' constituents depend upon the type of

mechanical behaviour that is being analysed. For instance, in addition to the finding that collagen fibres are the main load bearer during tensile loading, they have found that repetitive tensile loading affected their viscoelastic response.

Our use of fractional factorial design of experiments with ANOVA reduces the number of computational analyses required from full factorial and gives more statistical power. Since our R^2 values, which correspond to the proportion of the variability in the data explained by the ANOVA are all in the range of 77% - 92%, the ANOVA model seems to provide a good representation of the computational model. An improved fit may be obtained with a phenomenological constitutive model of increased complexity; however, it would lack the physical relevance of GOH. Another factor that is worth considering for better fits is water content variability during healing (Sharma and Maffulli 2006; Oakes 2008). It was reported that the content was high in the early stage of healing, and later reduced to its normal state to assist the maturation of scar tissue. Also, a model that formulates collagen variability and cellular activity during healing is expected to provide a more realistic representation, as these occurrences have been pronouncedly demonstrated in a study by Wu (Wu, Chen et al. 2010).

Another limitation is that the MATLAB optimisation scheme used (*lsqnonlin*) in the current study is sensitive to the initial values and can potentially identify local minima rather that the global one. This was controlled by manually testing all the fits with a large range of initial parameter values. They all resulted in the same final fitted values, thus we were fairly certain that identified values were of the global minima. However, other non-linear and constrained optimisation schemes should be tested in the future to ensure a robust search for the global optimum.

It is noteworthy that Design of Experiments is highly dependent on its parameter space (Montgomery 2006). As there are no previous literature reports of the GOH model in

tendon healing, we used ranges of values acquired from our own optimisation procedures. This requires the assumption that the experimental data used covered the parameter space comprehensively. The upper and lower bounds were set at two standard deviations, to ensure that the parameter space used was broad enough to avoid pre-determining the experiment.

Our study has focused on the behaviour of only the healing part of the tendon and neglected any changes in adjacent regions. There are no published data on the microstructure along the length of the tendon but such information will be essential for future mechanobiological studies of healing tendon.

In conclusion we have shown that a well established fibre-reinforced continuum model with distributed collagen orientations introduced by Gasser-Ogden-Holzapfel is able to simulate the mechanical behaviour of healing tendons. Good fits were obtained to mechanical tensile test data of healing tendons at day 3, 8, 14, 21 as well as the intact tissue. Our work further provides support for using the GOH fibre dispersion, κ, to represent collagen fibre organisation in healing tendons, and shows that this parameter, as well as the fibre stiffness at low strain and stiffening behaviour at large strain are most important under tensile load.

The work on the constitutive model described in this chapter is only the first step towards a full mechanobiological model of healing tendons. With the main objective of this thesis in mind (i.e. to develop computational approaches to enhance the knowledge of the role that mechanical factors play in fibre re-organisation in healing tendons), a model that is 'responsive' in a way is able to update its material properties depending on the surrounding biophysical stimuli as well as other mechanical factors is required. The next chapter presents the development of a mechano-adaptive model to simulate healing tendon, investigating the effect of different biophysical stimuli.

5 Mechano-adaptive models of healing tendons

This chapter presents the development of a mechanoregulatory framework and its application to healing tendons. As such, different biophysical stimuli, lengths of repair site and loading magnitudes were explored. Finite element analyses were performed using a hyperelastic fibre-reinforced continuum model with distributed collagen orientations, wherein the details of its execution were discussed in the previous chapter.

5.1 Introduction

Previous mechanobiological models were incorporating mechanoregulation rules, which were implemented within conceptual frameworks on skeletal mechanobiology (Carter, Beaupre et al. 1998). These frameworks, which explain the mechanical influences on skeletal tissue differentiation, were made by estimating the applied loads and inferring local stress and strain levels in the loaded tissue (Carter, Beaupre et al. 1998). Stress and strain are tensor quantities defined by specifying six components and a reference coordinate system. Pauwels (Pauwels 1960) proposed a scheme for differentiation of mesenchymal cells into musculoskeletal tissues, depending on the combination of volumetric and deviatoric deformation. Perren and Cordey (S.M. Perren and J. Cordey 1980) suggested tissue differentiation during bone healing was based on how much elongation each tissue type can tolerate. Claes and Heigele (Claes and Heigele 1999) proposed a model based on tensile strain and hydrostatic pressure with quantified thresholds (Fig. 5.1). Isaksson (Isaksson, Wilson et al. 2006) have later compared these schemes by using one computational model, and have suggested that the deviatoric stress may be the most significant mechanical parameter to guide tissue differentiation during indirect fracture healing. Most of these studies have used a poroelastic formulation to investigate both fluid and solid biophysical stimuli in the tissues. The formation of fibrous tissue was also addressed in these studies; however, the detailed microscopic representation of its collagen fibre re-organisation in response to biophysical stimuli has not been investigated.

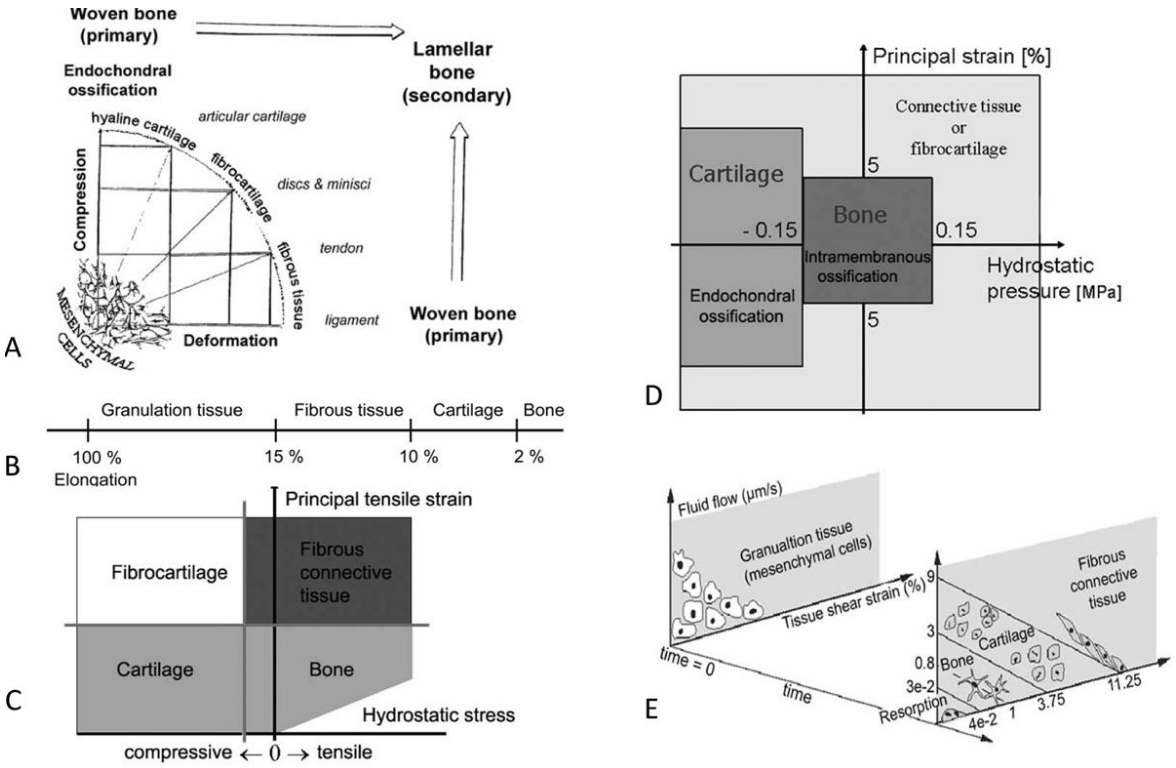


Figure 5.1. Previous mechanoregulatory algorithms for bone healing modelling, comparing effect of different biophysical stimuli, introduced by Pauwels (Pauwels 1960) (A), Perren and Cordey (S.M. Perren and J. Cordey 1980) (B), Carter (Carter, Beaupre et al. 1998) (C), Claes (Claes and Heigele 1999) (D), Prendergast (Prendergast 1997) (E). This figure is adapted based on Isaksson (Isaksson 2012) with permission.

Since collagen is the main load-bearing protein in tendons, it is speculated that healing tendon mechanics is highly influenced by collagen fibre re-organisation (Lanir 1983; Szczesny and Elliott 2014). A mathematical formulation for collagen fibre re-organisation has been well established (Baaijens, Bouten et al. 2010), and has been used to qualitatively simulate remodelling of fibres in soft tissues. For instance, simulation of fibre remodelling in cardiovascular tissues by Driessen (Driessen, Cox et al. 2008) obtained predictions comparable to experimental findings by including angled collagen fibres into the formulation to capture fibres that are not in principal direction (i.e. along the direction

of maximum principal tensile stress during normal physiological loading), for instance, the helical fibre structure. A further advance was to include fibre volume fraction, the ratio of fibre volume to total volume of the material, as a time dependent parameter, linked to fibre turnover for a fibre direction subject to a stretch, and rate of matrix protein synthesis (Driessen, Mol et al. 2007). This structural constitutive model of Driessen (Driessen, Mol et al. 2007) extends the model of Holzapfel (Holzapfel, Gasser et al. 2000) with a fibre volume fraction (van Oijen 2003) to describe the relative amount of fibres present in each direction. All these aforementioned models are phenomenological, provide qualitative predictions and have not been compared against quantifiable structural parameters (Baaijens, Bouten et al. 2010), mainly due to lack of experimental information.

A constitutive model with a combination of phenomenological and micro-structural parameters has recently been used to capture healing tendons mechanics at specific timepoints, as described in Chapter 4 (Bajuri, Isaksson et al. 2016). It used a hyperelastic fibre-reinforced continuum model with distributed collagen fibre orientations introduced by Gasser-Ogden-Holzafel (GOH) (Gasser, Ogden et al. 2006) for the constitutive formulation. This model was selected as some of its parameters are tied to physical representations. Other more complex models often have many more parameters with low physical meaning which are then difficult to quantify experimentally. Apart from suggesting the use of this model for healing tendon mechanics simulation, the previous chapter identified that the representation of collagen fibres in this model was most important for the overall mechanical response, and that the representation of non-collagenous matrix played a minor role. Chapter 4 also demonstrated the use of the model in two-dimensional finite element analysis (FEA), which is important to assess the spatial distribution, especially in the context of fibre re-organisation across the tissue during different stages of healing. Experimental studies show that the angular distribution of

fibres is important and varies highly both temporally and spatially during the repair process (Sasaki, Yamamoto et al. 2012; Nourissat, Berenbaum et al. 2015). It is therefore speculated that this model could be used to simulate mechano-adaptation of healing tendons, and yet up until now no such effort has been made.

The aim of this study is to propose a computational platform for adaptive simulations of tendon healing by implementing a model that uses a change in fibre dispersion to represent tissue reorganisation. Furthermore, it aims to test its sensitivity to variation in callus geometry and loading parameters and to evaluate three possible biophysical stimuli. The platform was based on the use of the finite element method (FEM) with the GOH constitutive model including fibre orientation and dispersion. FEA was used to calculate thresholds for tissue types' turnover, and then to capture spatial distribution of fibre re-organisation across the tissue during healing progression. The predicted material properties and spatial distributions from the simulations were compared qualitatively with previously published experimental data (Schepull, Kvist et al. 2007; Rosenbaum, Wicker et al. 2010; Sasaki, Yamamoto et al. 2012; Connizzo, Yannascoli et al. 2013).

5.2 Materials and methods

5.2.1 Finite element models

The development of the computational models was based on two important studies. Firstly, an experimental study using a rat model of Achilles tendon healing in loaded and unloaded conditions by Eliasson (Eliasson, Andersson et al. 2009), was used to develop the geometry and material properties, i.e. length and cross sectional areas. Eliasson et al. performed tensile testing of the healing tendons at days 3, 7, 14 and 21 after transection. The constitutive parameters used in this thesis were estimated from their data. Secondly,

an experimental study that employed scanning electron microscopy (SEM) to determine the collagen fibre organisation in a rat model of healing tendons, at days 7, 14, 28, 56 and 180 after transection (Sasaki, Yamamoto et al. 2012) was used to motivate the suggested fibre dispersion change over time.

Axisymmetric FE models of rat Achilles tendons with two regions - tendon stump and repair site were created as shown in Figure 5.2. The length (2.44 mm stump, 4.50 mm repair site) and width (0.55mm) of the models were half of the average values reported experimentally (Eliasson, Andersson et al. 2009) since symmetry boundary conditions were used. The model geometry remained constant over time. Reduced integration hybrid rectangular axisymmetric (CAX8RH) elements were used. Following a convergence study (Fig. 5.3), a mesh size of 0.04 mm was chosen. Force was applied to the nodes on the top edge, with translation allowed only in *y* direction, and all rotational degrees of freedom of the nodes were constrained (Fig. 5.3). Symmetric boundary conditions were applied to the other two edges – xymm and ysymm. The model was run as a static analysis in ABAQUS/Standard (v6.12-4, Dassult Systemes, France) using the NLGEOM solver to account for large deformation of the tendon.

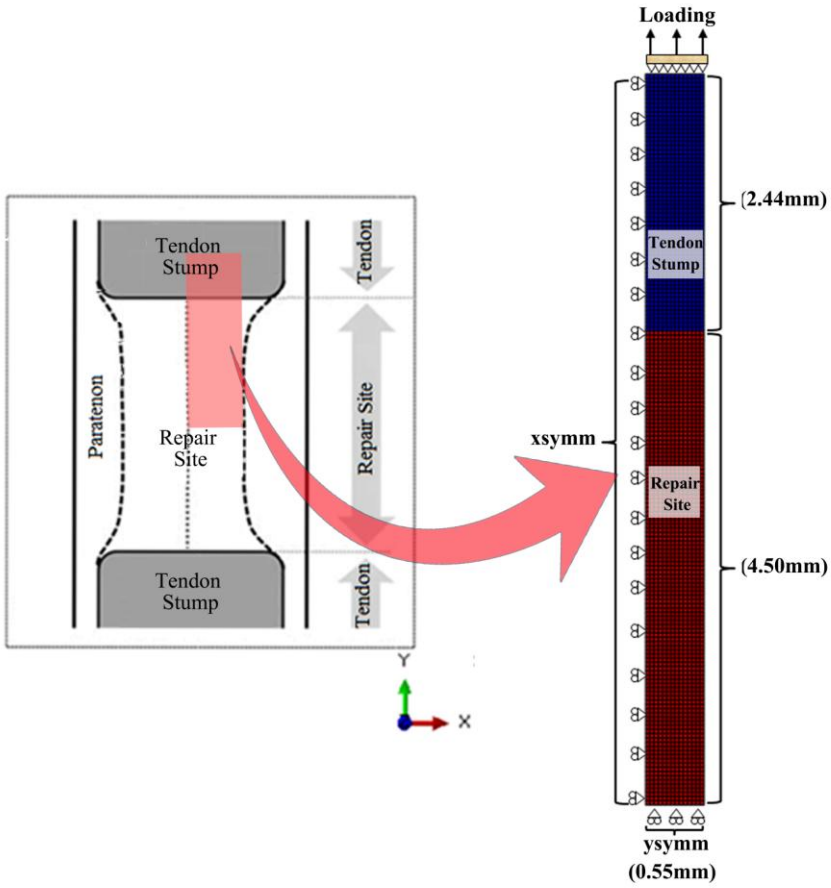


Figure 5.2. Dimensions of the simulated tendon (axisymmetric). xsymm and ysymm indicate that these edges are the symmetrical line of the body with respect to its corresponding axis and are constrained in that particular direction. For instance, the xsymm edge was constrained in the *x* direction, which only allows the body to move in the *y* direction.

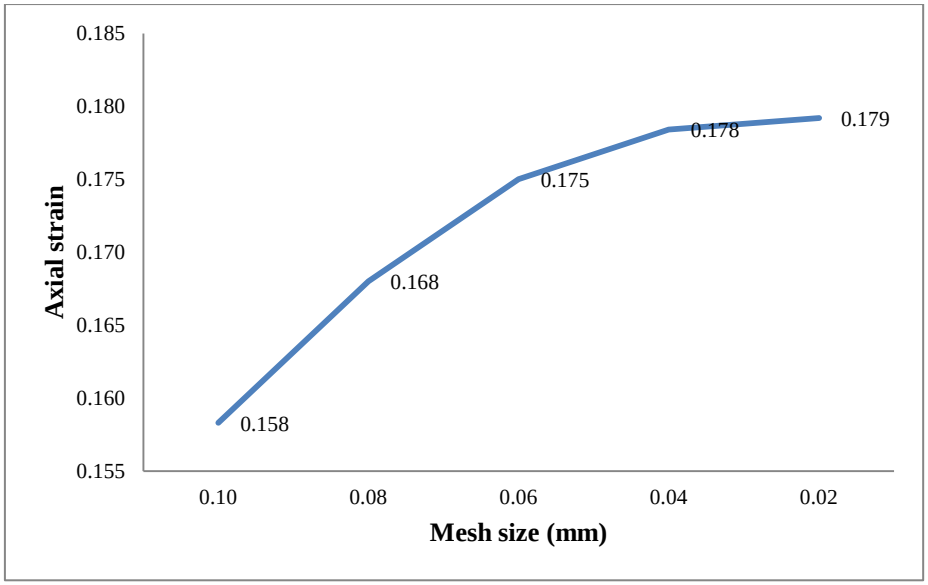


Figure 5.3. Grid-convergence test. For testing convergence, five finite element studies were performed with varied element sizes; 0.10, 0.08, 0.06, 0.04, and 0.02 mm. The axial strain was evaluated in elements located directly under the tendon stump (2 mm starting from the tendon stump to the repair site) where the average strain values were calculated. There were only small differences in the strain values between the 0.04 mesh-size study and the 0.02 mesh-size study, hence the former was selected throughout the study, as it results in lesser computational complexity compared with the latter.

5.2.2 Constitutive model

The GOH constitutive model used parameters that were quantified using the same model fitting scheme reported in Chapter 4 (Bajuri, Isaksson et al. 2016), while the computational works used the GOH model implementation included in a finite element analysis software, ABAQUS/Standard (v6.12-4, Dassault Systèmes, France).

Four different tissue types were defined with different degrees of tissue orientation. The fibre re-organisation that occurs during healing was represented by changing the tissue type assigned to each element. The tissue types, namely F0, disorganised; F1, partially organised; F2, moderately organised; F3, well-organised, were based on histological

scoring of healing tendons (Rosenbaum, Wicker et al. 2010). F0, F1, F2, F3 were assumed to correspond approximately to day 3, 8, 14, 21, respectively in the rat model of healing tendon reported by Eliasson (Eliasson, Andersson et al. 2009) and these data were used to quantify material properties. Intact tendon properties were used to characterise the tendon stump. Using the SEM images as a guide (Sasaki, Yamamoto et al. 2012), the fibre dispersion values, κ for each tissue type were set to 0.25, 0.23, 0.21, 0.19 and 0.00, respectively. The fitted constitutive parameters for each fibre structure were as shown in the Table 5.1. The non-monotonic changes of quantified values observed are likely due to the combined influence of changes in material property, callus geometry and composition (Bajuri, Isaksson et al. 2016). The fibre preferred direction, γ, was set to 0^o (along the loading direction).

Table 5.1 Parameter values and input used in the finite element analyses

Tissue Types	k_1'	k_2'	C_{10}
F0	2.53	2.59	0.746
F1	3.67	0.90	0.809
F2	7.52	0.70	1.228
F3	5.25	1.05	1.436
Stump	7.30	0.75	1.139

5.2.3 Thresholds selection for fibre re-organisations

Following Perren (S.M. Perren and J. Cordey 1980), a mechanoregulatory scheme was adopted that, for the same stress, proposed tissue types with higher stiffness could only form at lower strain (Fig. 5.4). This scheme is also suggested by more recent work showing tenogenic differentiation requires a stiffer matrix/substrate than granulation tissue (Sharma and Snedeker 2010).

Following Claes (Claes, Heigele et al. 1998) force control models were used and three strain-based scalars were chosen for investigation as biophysical stimuli: tendon axial strain (S1), principal strain (S2) and deviatoric strain (S3). Single phase models were used so poroelastic stimuli could not be investigated. Calculations of thresholds for the transition from one tissue type to another were performed using FEM for each biophysical stimulus, wherein average values were measured and assumed to be representative of those thresholds (Table 5.2). It should be pointed out that these average values were of maximum numbers (e.g. maximum principal strain), which is similar to an approach used for thresholds quantification by Claes (Claes and Heigele 1999). Tissue type change was permitted only in one direction so elements did not reduce stiffness if strains increased (Fig. 5.4b). The material properties used were as shown in Table 5.1. An axial load of 1.6N was applied, based on measurements of force transferred from soleus and gastrocnemius muscles to calcaneus bone through the rat Achilles tendon (Winiarski, Roy et al. 1987; Szaro, Witkowski et al. 2009).

Adaptive models of bone healing have suggested principal strain (S2) thresholds for fibrous tissue formation of 5% (Carter, Beaupre et al. 1998) and 15% (Claes and Heigele 1999) and deviatoric strain threshold (S3) of 5% (Isaksson, Wilson et al. 2006). Axial strain (S1) threshold lies in a range of 10 – 15% (S.M. Perren and J. Cordey 1980). Proposed thresholds in the present study were within these ranges (Table 5.3).

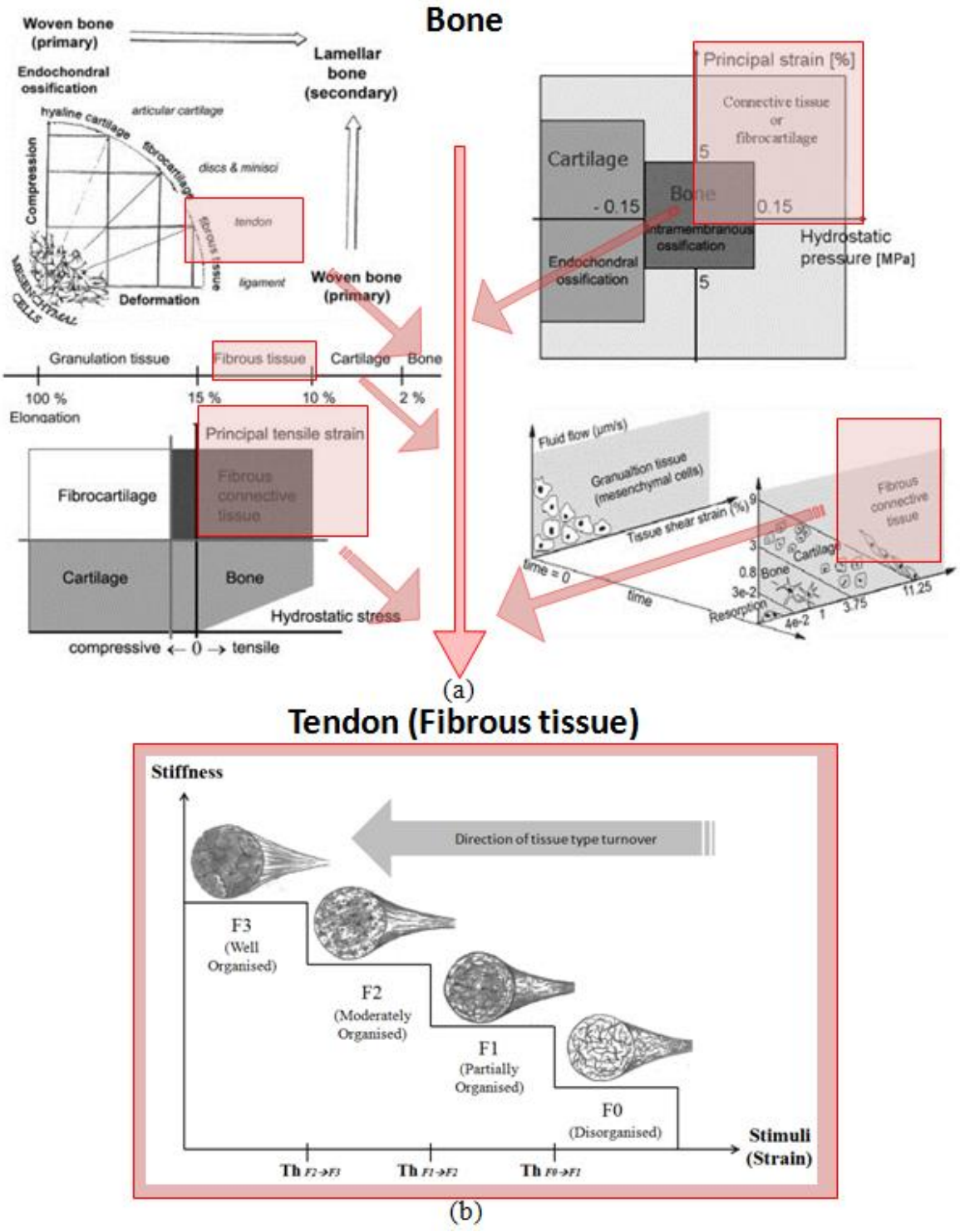


Figure 5.4. The proposed mechanoregulation scheme for tendon healing (b), as compared to previous schemes for bone healing (a). Stimuli magnitudes increase from left to right of the axis (b). Only strain components of the previous scheme were considered in this study. Parts of this figure were adapted from Isaksson (Isaksson 2012) with permission. '**Th'** stands for thresholds for tissue type turnover

Table 5.2. The measured thresholds values for models subjected to 1.6N

Tissue type turnover	Biophysical stimuli		
	Axial (S1)	Principal (S2)	Deviatoric (S3)
F0→F1	0.21	0.21	0.15
F1→F2	0.16	0.16	0.12
F2→F3	0.09	0.09	0.06

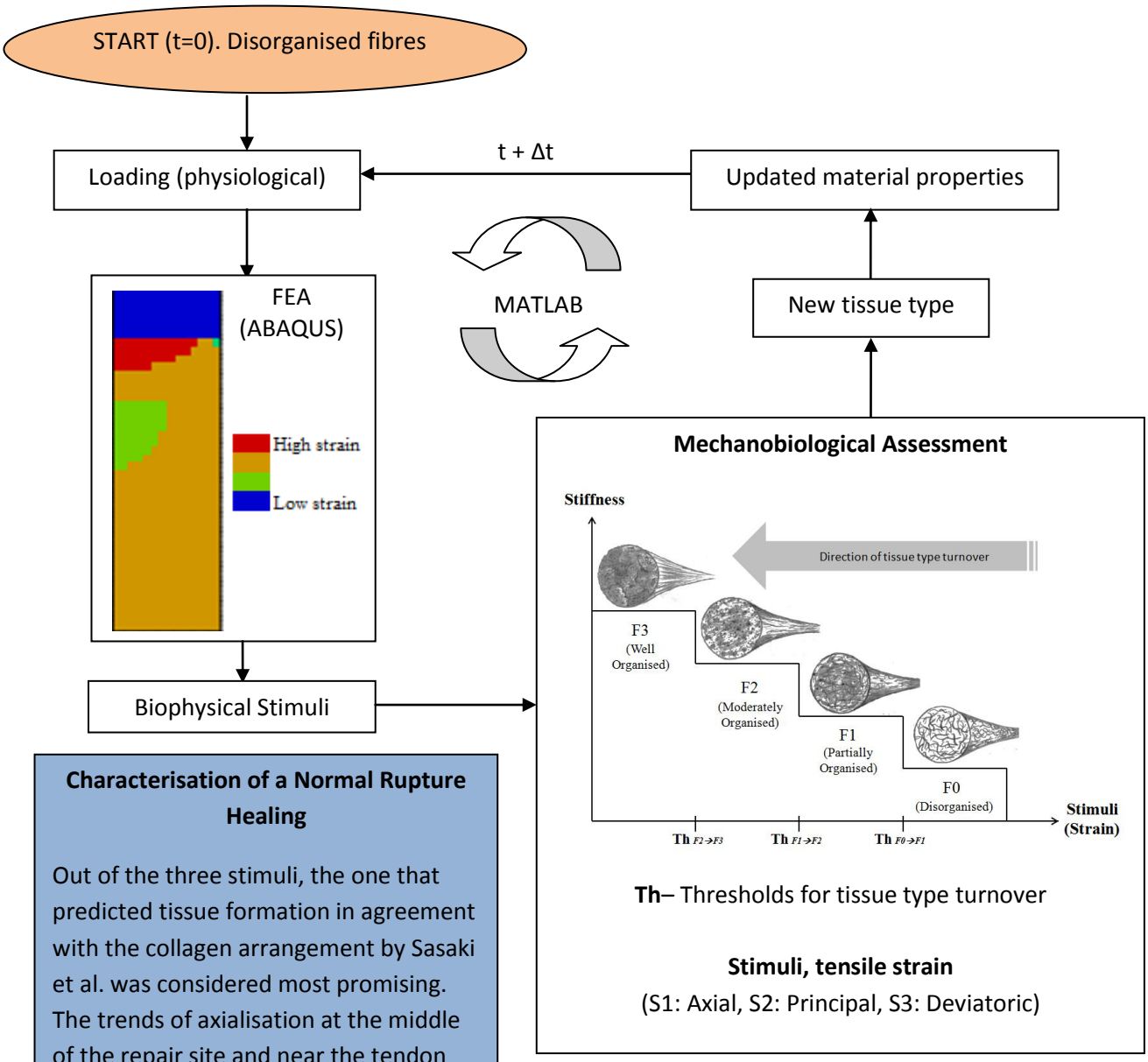
Table 5.3. A comparison between thresholds obtained in this study and ones reported in the literature. Please note the thresholds were of average of all cases (all loading magnitudes and length of the models, wherein these changes were part of sensitivity analyses, discussed further in Section 5.2.6)

Fibre turnover	Thresholds for fibrous tissue formation as reported in literature	This study	Within range?
Axial (S1) & Principal (S2) Strain (%)			
F0→F1	> **10 - 15** for axial (S.M. Perren and J. Cordey 1980) and > **5 or 15** for principal (Carter, Beaupre et al. 1998; Claes and Heigele 1999)	28	Yes
F1→F2		22	Yes
F2→F3		14	Yes
Deviatoric (S3) Strain (%)			
F0→F1	> **5** (Isaksson, Wilson et al. 2006)	20	Yes
F1→F2		16	Yes
F2→F3		10	Yes

5.2.4 Mechanoregulatory algorithm

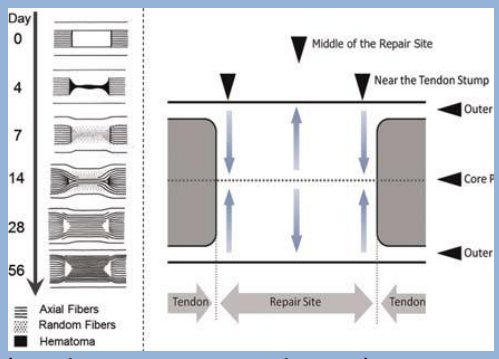
The iterative procedure to simulate mechano-adaptive behaviours of healing tendons, starts with a strain analysis in ABAQUS using FE method (Fig. 5.5). Biophysical stimuli were calculated at maximum loading and the output from the FE analyses was passed to MATLAB for mechanoregulation assessment. The MATLAB script was run to determine new material properties for each element, according to the proposed mechanoregulation rule. If the stimulus for the element fell below the defined threshold for change from its current tissue type, its tissue type was changed and its material properties were updated. These properties were then used as input for the FE analyses in ABAQUS in the next iteration. The simulation ran until a steady state, which is when no tissue turnover or healing progression, takes place. An arbitrary initial number of iterations, 35 was set to all simulations. Greater numbers of iterations were performed if the simulation had not reached a steady state within 35 iterations. Complete healing was defined when all elements in the model had been assigned tissue type F3 – well organised tissue type properties. A stimulus was counted to favour healing when fibre re-reorganisation progressed to completion following the right sequence of tissue type turnover (Fig. 5.4b). The speed of progression to the healed state was assessed separately. Inhibition of healing was defined when a stimulus caused the fibre re-organisation to stop progressing before completion, and remained not changing after at least 20 iterations.

This study focused on fibre re-organisation during healing and hence the early granulation stage was neglected. The repair site was initially set to consist of disorganised fibre (F0). Only the repair site was allowed to update its material properties while the tendon stumps remained unchanged. Outcomes of each analysis were assessed by comparing temporal and spatial distributions of tissue type and overall model stiffness with experimental findings (see Section 5.2.5 for more details).



Characterisation of a Normal Rupture Healing

Out of the three stimuli, the one that predicted tissue formation in agreement with the collagen arrangement by Sasaki et al. was considered most promising. The trends of axialisation at the middle of the repair site and near the tendon were completely opposite. Near the tendon stump, the progression starts from outer layer then towards the core. On the contrary, in the middle of the repair site, it progresses from the core to the outer layer.



(Sasaki, Yamamoto et al. 2012)

Figure 5.5 Rupture healing in mechanoregulated adaptive model in MATLAB. The iterative procedure starts with a strain analysis in ABAQUS, where the biophysical stimuli for each stimulus are calculated. The fibre structure is determined for each element based on the proposed mechanoregulation rule, and the tissue properties are updated before the next iteration begins.

5.2.5 Outcome assessments

Comparative analyses were performed qualitatively in which distributions of tissue type during healing, characterised by the fibre dispersion, κ value were compared with studies of tissue organisation during tendon healing (Rosenbaum, Wicker et al. 2010; Sasaki, Yamamoto et al. 2012; Connizzo, Yannascoli et al. 2013). Changes of material properties (i.e. stiffness) during healing were quantitatively compared with previous experimental works (Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Andersson, Eliasson et al. 2012). These studies have generally reported that stiffness of healing tendons increases over time, and when subjected to higher loading magnitudes.

5.2.6 Sensitivity analyses

The physiological loads in rat Achilles are known to vary between 1.0 – 2.2 N (Winiarski, Roy et al. 1987; Szaro, Witkowski et al. 2009). Further, although the adaptive model presented here has a fixed length, the rat Achilles tendon changes length during the healing process, from 3.0 – 15.0 mm (Eliasson, Andersson et al. 2009). Therefore, a set of sensitivity studies was carried out to explore these parameter spaces (Table 5.4). For each combination of these parameters an adaptive model was run. In total, there were 75 analyses performed.

Please note the different settings used in the sensitivity analyses resulted in different thresholds as well. Thus, they were recalculated using FEA, prior to the adaptive analysis. All thresholds fell within the ranges defined by previous authors as stimulating fibrous tissue formation (Table 5.2). The changes of thresholds for tissue turnover, F0→F1 with respect to different loading magnitudes and lengths of the model are as shown in Table 5.5. The change of the thresholds due to the change of length from 1.5 to 7.5 mm, increases with loading magnitude (Fig. 5.6a). In contrast, the change of the thresholds due

to the change of loading magnitude from 1.0 to 2.2 N, decreases with the length of the model (Fig. 5.6b). These changes apply to all stimuli. The overall flow of the work conducted in this chapter, which included sensitivity analyses, is as shown in Figure 5.7.

Table 5.4 Parameter space for the sensitivity analyses

Parameters		
Length (mm)	**Load (N)**	**Stimuli**
1.5	1.0	S1
		S2
		S3
	1.3	S1
		S2
		S3
	1.6	S1
		S2
		S3
	1.9	S1
		S2
		S3
	2.2	S1
		S2
		S3

A combination of these three parameters was counted as one analysis. In total, there were 75 analyses

Same setting applies for models of different lengths (3.0, 4.5, 6.0, 7.5mm)

Parameters		
Length (mm)	**Load (N)**	**Stimuli**
7.5	1.0	S1
		S2
		S3
	1.3	S1
		S2
		S3
	1.6	S1
		S2
		S3
	1.9	S1
		S2
		S3
	2.2	S1
		S2
		S3

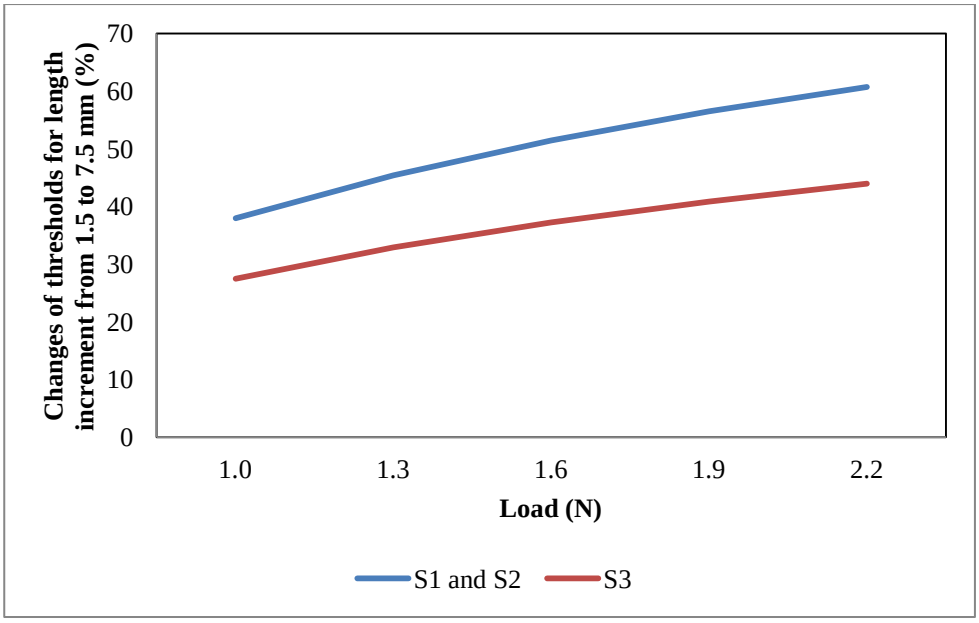
Table 5.5 Recalibrated thresholds for tissue type turnover F0→F1, with respect to changes of loading magnitude and lengths for axial and principal strain (a) and deviatoric strain (b).

F0→F1	Axial (S1) and principal (S2) strain (%) Load (N)					Changes of thresholds for load increment from 1.0 to 2.2 N (%)
Length (mm)	**1.0**	**1.3**	**1.6**	**1.9**	**2.2**	
1.5	46	56	63	70	75	29
3.0	22	27	32	35	39	16
4.5	15	18	21	23	26	11
6.0	10	13	15	17	18	8
7.5	8	10	12	13	15	6
Changes of thresholds for length increment from 1.5 to 7.5 mm (%)	38	45	51	56	61	

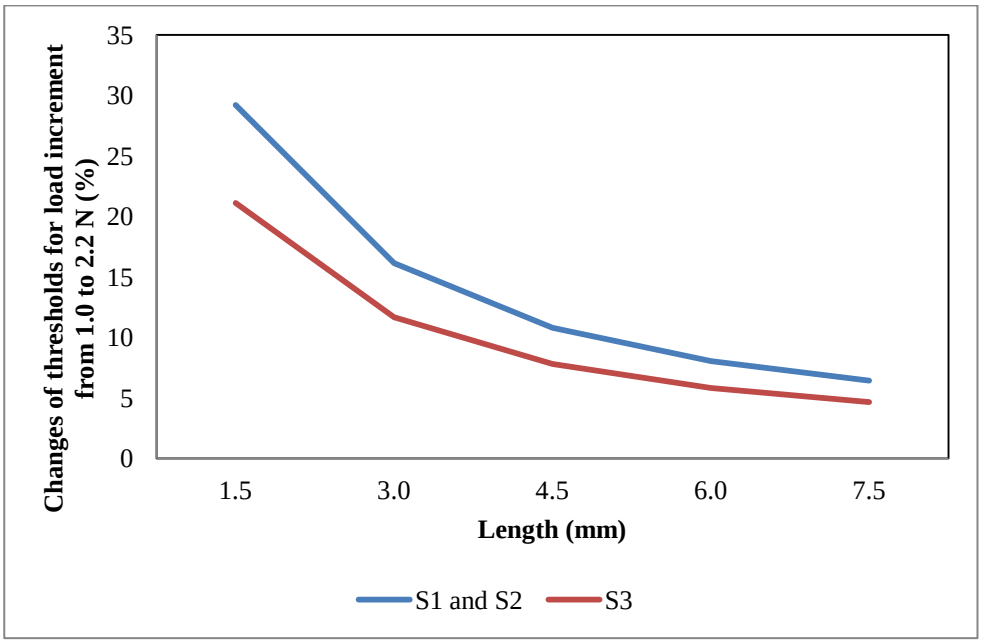
(a)

F0→F1	Deviatoric (S3) strain (%) Load (N)					Changes of thresholds for load increment from 1.0 to 2.2 N (%)
Length (mm)	**1.0**	**1.3**	**1.6**	**1.9**	**2.2**	
1.5	33	40	46	51	55	21
3.0	16	20	23	26	28	12
4.5	11	13	15	17	18	8
6.0	8	9	11	12	13	6
7.5	6	7	9	10	11	5
Changes of thresholds for length increment from 1.5 to 7.5 mm (%)	27	33	37	41	44	

(b)



(a)



(b)

Figure 5.6. Changes of thresholds for tissue type F0→F1 turnover for length increment from 1.5 to 7.5 mm, with respect to change of loading magnitude (a) and for load increment from 1.0 to 2.2 N, with respect to change of length (b). Please note the exact values were as shown in Table 5.5.

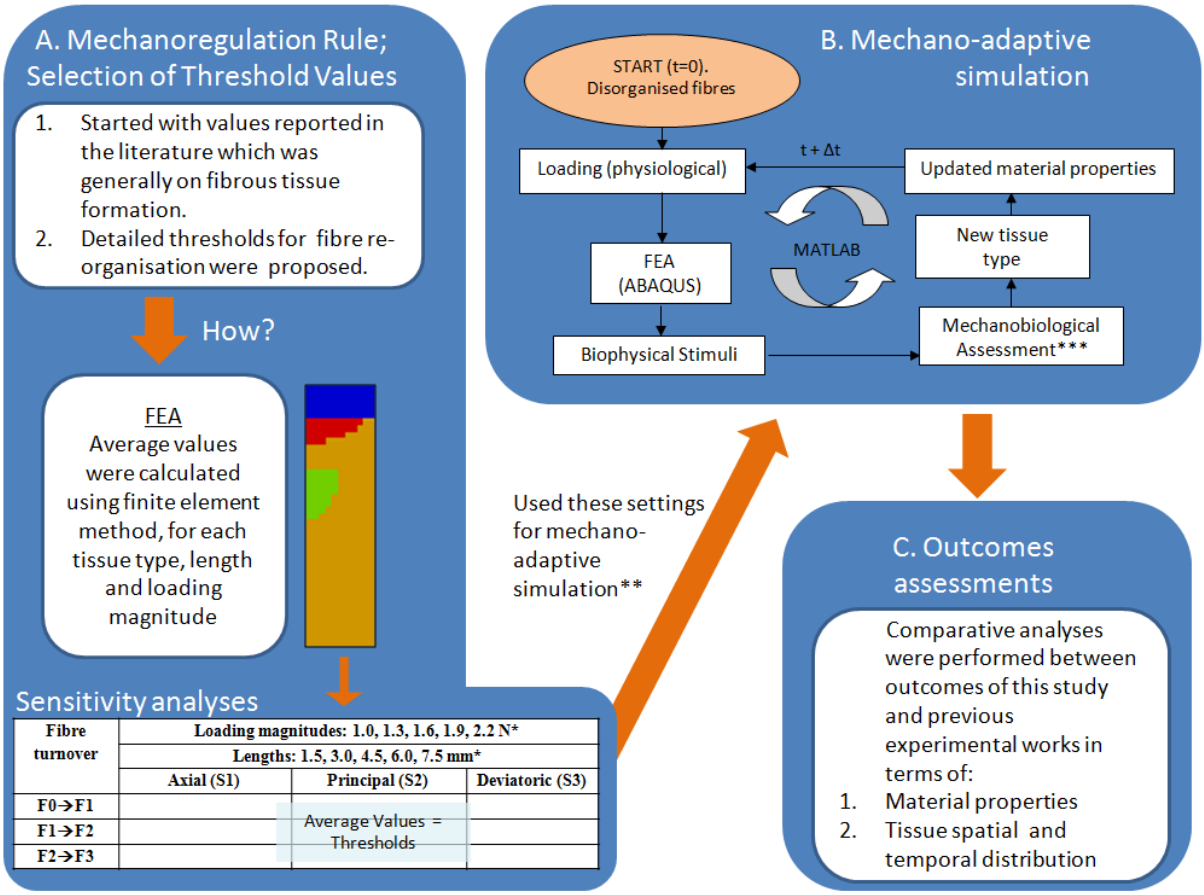


Figure 5.7. The flowchart of the works performed in this study. The proposed mechanoregulation rule was first detailed in which thresholds for tissue type change were calculated using FEA (A). Effects of these thresholds were tested in mechano-adaptive simulations of healing tendons, for each biophysical stimulus, length and loading magnitude (B). Comparative analyses were then performed with material properties and tissue spatial and temporal distributions from previous reports (C). Please note that (*) signifies these values were analysed using separate models and respective values were calculated separately. (**) indicates mechano-adaptive simulations were performed separately for each combinations of parameters (75 simulations performed). (***) indicates a point where mechanoregulation rule with its proposed thresholds was applied.

5.3 Results

5.3.1 Model stiffness

Generally, the stiffness of the models regulated by all stimuli, regardless of lengths and loading magnitudes increased with increasing number of iterations. They all started with a similar initial stiffness and all reached the same maximum stiffness (Fig. 5.8). Moreover, the models regulated by S1 and S2 generally completed the healing faster than the models regulated by S3, at iteration 16 and 65, respectively.

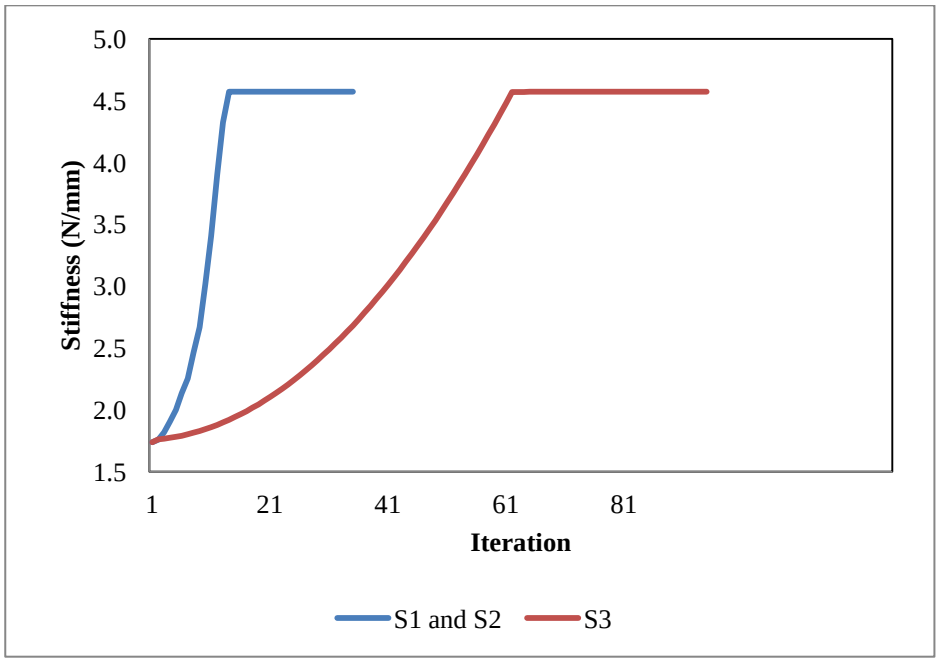


Figure 5.8. Stiffness of models regulated by S1 and S2, and by S3 over iterations. The reported stiffness values were of average of all lengths and loading magnitudes

5.3.2 Fibre re-organisation regulated by axial strain (S1), principal strain (S2) and deviatoric strain (S3)

In general, the change in tissue type regulated by S1 and S2 produced similar outcomes both spatially and temporally, and the predictions were distinctly different from those predicted by S3.

Simulations using S1 and S2 as mechanical signals showed tissue change starting on the outer layer of the repair site near the tendon stump (Fig. 5.9). In these simulations a flipping instability between the outer layer and core region was observed, which continued as tissue change progressed from the tendon stump to the middle of the repair site, until complete healing (Fig. 5.10).

Different patterns of tissue adaptation were observed for models regulated by S3 (Fig. 5.9 and 5.11). With S3, the progression started near the core region near the tendon stump, and progressed to the middle of the repair site until complete healing was achieved. These models required up to 5 times the number of iterations to complete healing, compared to those regulated by S1 and S2 models. For instance, a model with a dimension of 6 mm and subjected to 1.6 N required 88 iterations to complete healing, as compared to only 17 iterations for S1 and S2 regulated models of the same length and loading magnitude (Fig. 5.12).

5.3.3 Effect of different lengths and loading magnitudes on the models of different stimuli regulation

Different lengths - Models regulated by S3 were more sensitive to changes of length than S1and S2. Short S3 models, 1.5, 3.0 and 4.5 mm, inhibited healing when progression stopped at an average of 7, 19, and 51 iterations, respectively, whilst its longer models (6.0 and 7.5 mm) favoured healing when simulations were in average completed at iteration 65 (Fig. 5.12). These effects were not seen in S1 and S2 models wherein models of all lengths were found favouring healing.

In general, longer models progressed to completion more quickly than shorter models (Fig. 5.12). For instance, S1 and S2 models with a length of 7.5 mm required on average 16 iterations to complete compared with 36 iterations for models with a length of

1.5 mm. S3 models with a length of 7.5 mm required only 42 iterations compared with the model of 6.0 mm length, which needed 88 iterations to complete.

Different loading magnitudes - Models regulated by S3 were more affected by changing loading magnitudes as compared to models regulated by S1and S2 (Fig. 5.10 and 5.11). Higher magnitude caused S3 models to have lesser more-organised tissue type turnover across the model (Fig. 5.11). For instance, at iteration 30, only half of the model subjected to 1.3 N (length of 6.0 mm) consisted of F3 (well-organised) tissue type while the same model subjected to lower loading magnitude of 1.0 N had nearly three quarters of the F3 tissue type. This effect of spatial distribution was less discernible in models regulated by S1 and S2 (Fig 5.10).

Higher loading magnitudes also caused longer S3 models (6.0 and 7.5 mm) to require more iterations to complete healing (Fig. 5.13). For instance, S3 models subjected to 1.9 N required 94 iterations to complete, while the same model subjected to 1.6 N required only 81 iterations. Models regulated by S1 and S2 showed no such differences especially for longer models (6.0, 7.5 mm) (Fig. 5.10). But for shorter models (1.5, 3.0 and 4.5 mm), higher loading magnitudes caused the models to complete faster, especially the model of a length of 4.5 mm (Fig. 5.13). In other words, higher tensile load somewhat favoured fibre re-organisation in S1 and S2 models, but not S3 models.

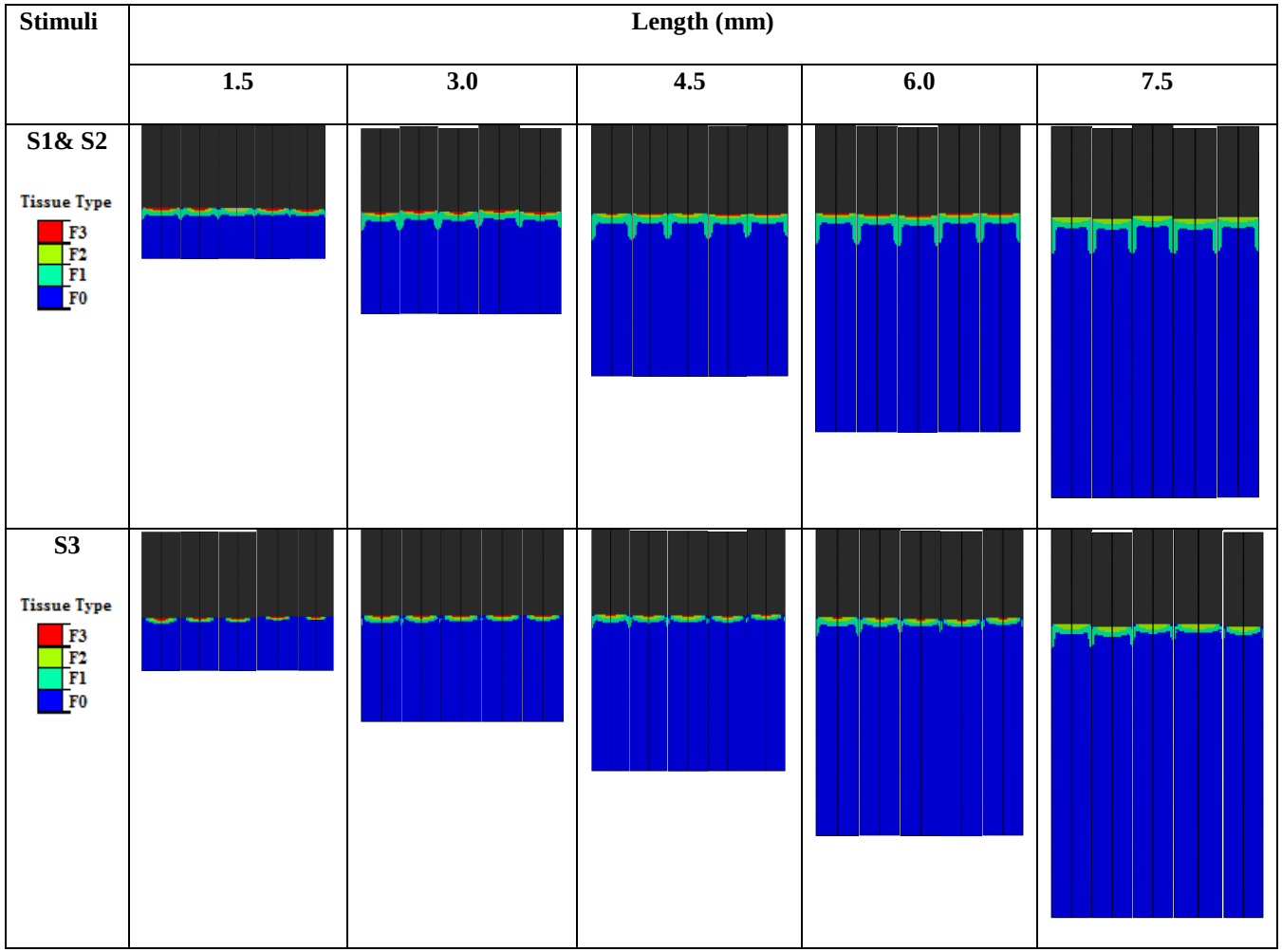


Figure. 5.9. Fibre organisation for different lengths and loading magnitudes with respect to different mechanical stimuli for iteration 2 (early healing). Please note that the 5 models in each square indicate models subjected to loading magnitudes of 1.0, 1.3, 1.6, 1.9 and 2.2N, respectively, and the legend illustrates tissue types, F3, F2, F1 and F0 represent well-organised, moderately organised, partially organised, and disorganised, respectively

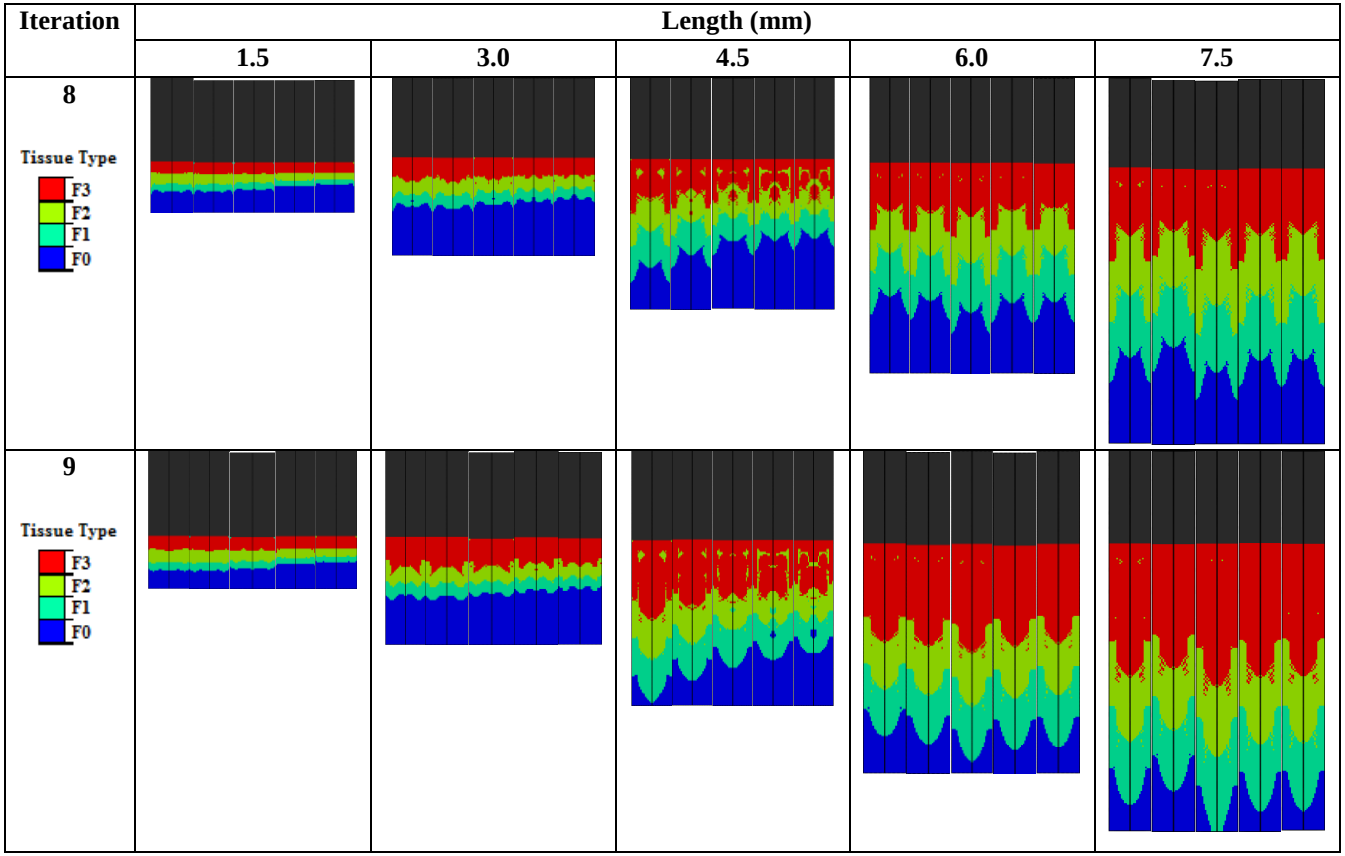
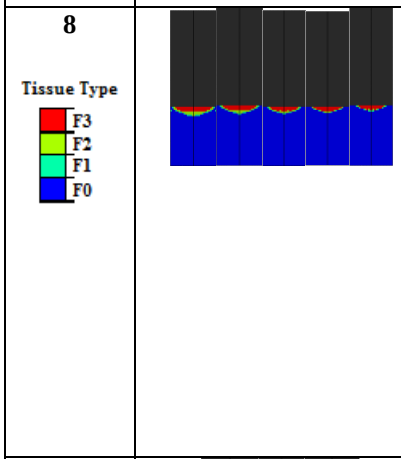
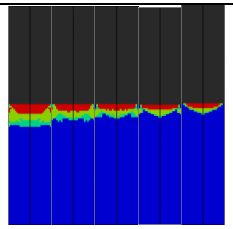
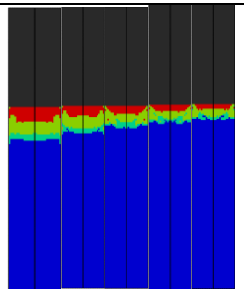
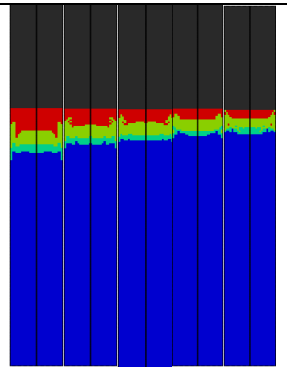
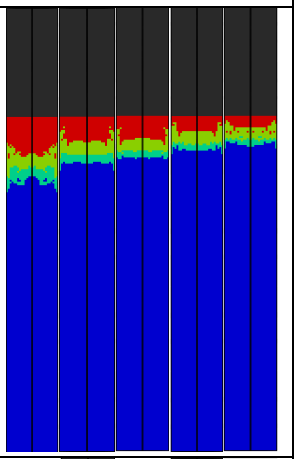
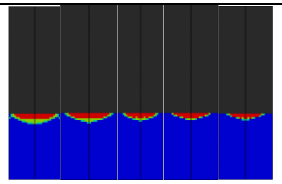
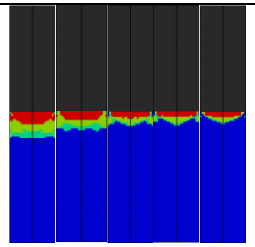
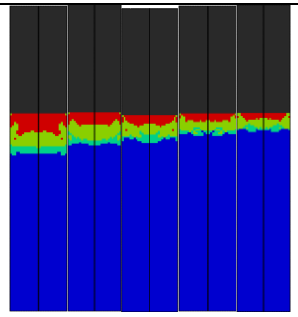
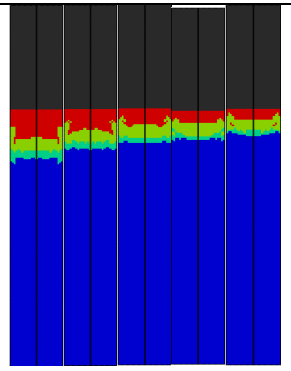
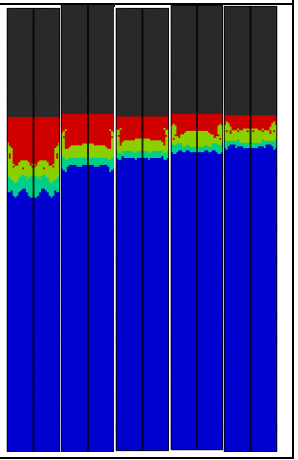


Figure 5.10. Fibre organisation for different lengths and loading magnitudes for models regulated by the axial (S1) and principal (S2) strain, for iteration 8 and 9 (along the process of healing). The "flipping pattern" of the predicted tissue at the boundaries as seen at iteration 8 and 9 was observed until healing completed. Please note that the 5 models in each square indicate models subjected to loading magnitudes of 1.0, 1.3, 1.6, 1.9 and 2.2N, respectively, and the legend illustrates tissue types, F3, F2, F1and F0 represent well-organised, moderately organised, partially organised, and disorganised, respectively.

Iteration	Length (mm)				
	1.5	3.0	4.5	6.0	7.5
8					
9					

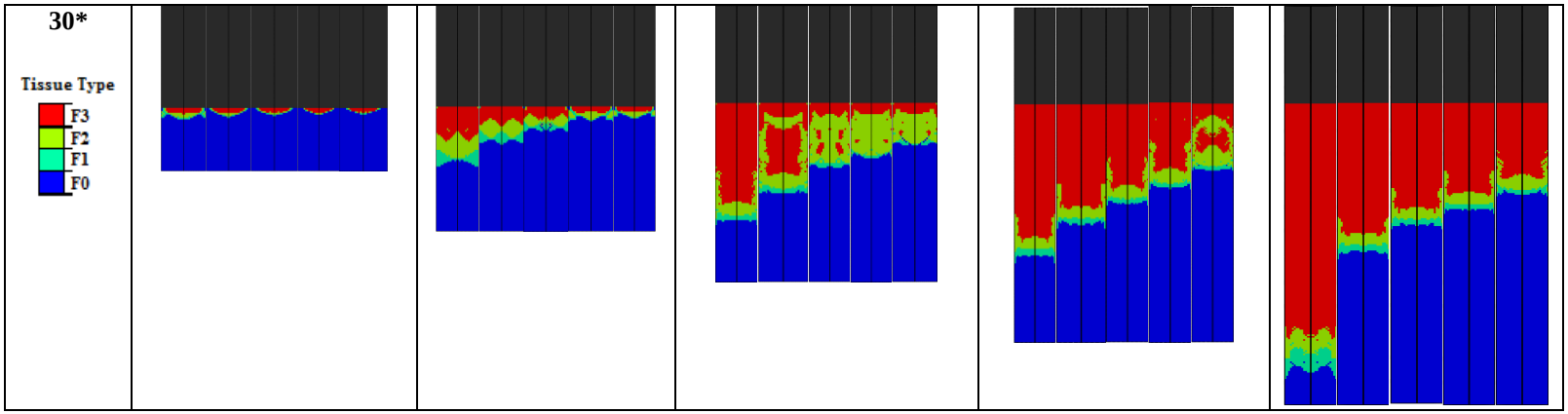


Figure 5.11. Fibre organisation for different lengths and loading magnitudes of models regulated by the deviatoric strain (S3), for iteration 8, 9 and 30 (along the process of healing). Please note that the 5 models in each square indicate models subjected to loading magnitudes of 1.0, 1.3, 1.6, 1.9 and 2.2N, respectively, and the legend illustrates tissue types, F3, F2, F1 and F0 represent well-organised, moderately organised, partially organised, and disorganised, respectively. Please note, extra tissue distribution at iteration 30 with this symbol (*), was included for models regulated by S3 but not for S1 and S2 due to more iterations were required for completion. This additional iteration was to ensure clearer differences in terms of spatial distribution could be observed.

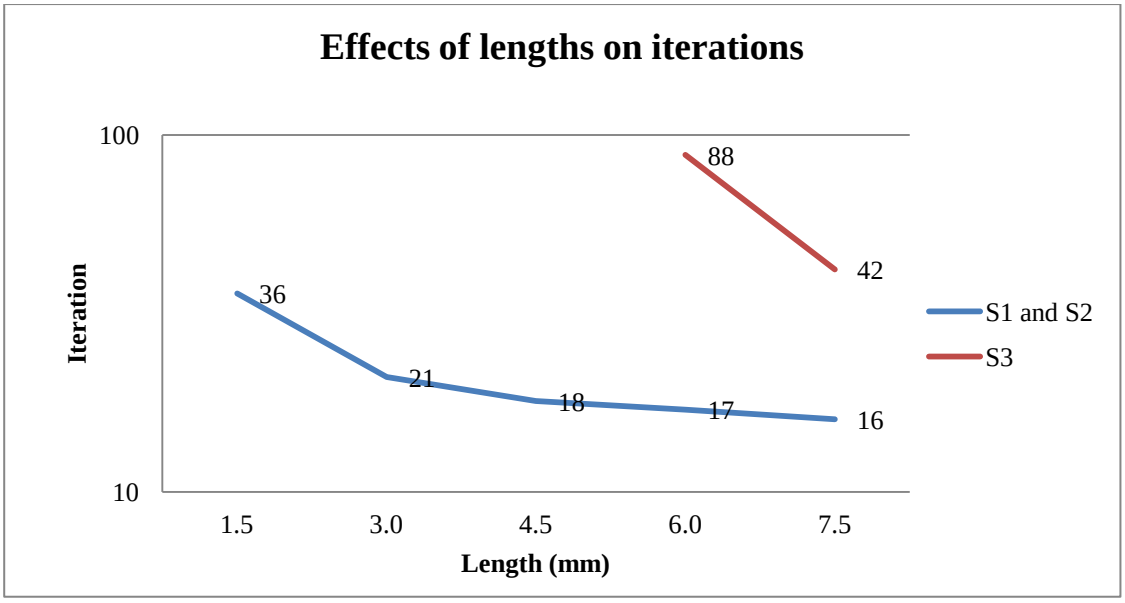


Figure 5.12. Time to complete healing for models with different lengths when regulated by S1, S2 and S3, and subjected to 1.6 N load magnitude. Please note that there are no iteration values for short models regulated by S3 due to simulations not being completed i.e. achieved steady state that was not complete healing.

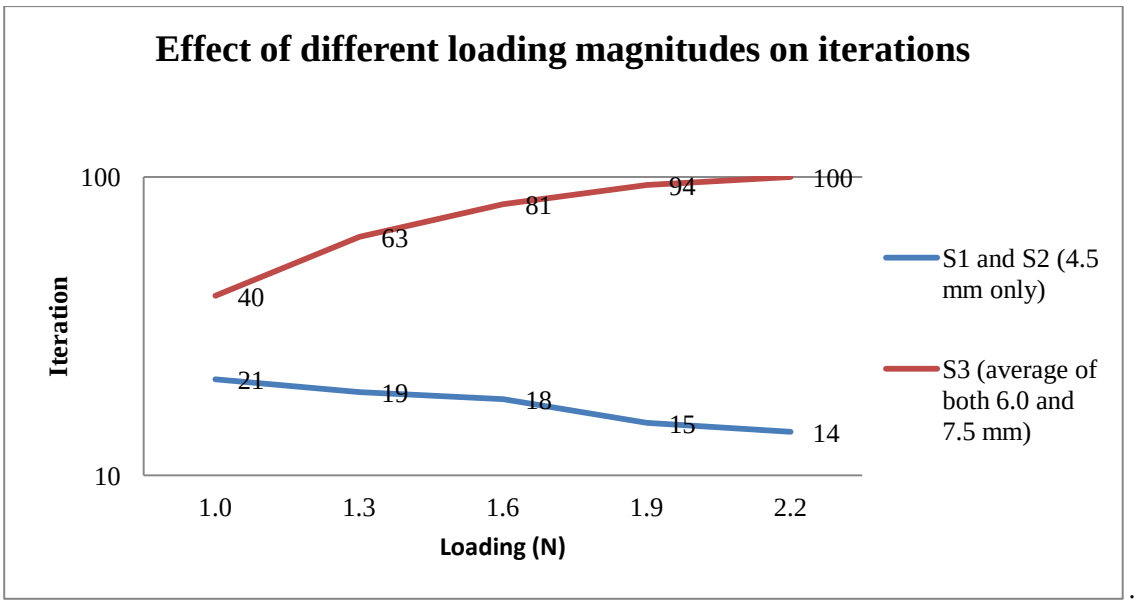


Figure 5.13. Time to complete healing with respect to different loading magnitudes.

5.4. Discussion

Computational modelling of mechanobiology can provide critical insight into the factors that enhance or inhibit tendon healing when used alongside in vivo models and hence can provide the inspiration for new treatments and therapy approaches (Thompson 2013). In this study, a first simplified computational method that can simulate the change in tissue type from disorganised to more organised collagen fibres has been developed and applied to the problem of healing tendons. To the best of our knowledge, it is the first reported use of finite element analysis to simulate mechanically mediated tendon regeneration, particularly by different strain tensor components. Predicted fibre re-organisation patterns and changes in material properties over time generally accord with those observed experimentally. This study suggested that the axial and principal strain (S1 and S2, respectively) are superior to deviatoric strain (S3) in regulating healing tendons fibre re-organisation, but still there is more work to be carried out in order to validate these findings, as discussed later in this section.

The increased stiffness that was predicted during healing by all models was in agreement with experimental findings (Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Andersson, Eliasson et al. 2012). The values of the stiffness were within the reported range (Fig. 5.8). The minimum and maximum values were similar for all stimuli; with the only difference being the time to complete healing. In this study, the simulated temporal distribution was not well established (i.e. no parameters related to time during adaptive process were included) as discussed later in this section, hence simply looking at the time of healing for a definite comparison is insufficient. In fact, these findings further emphasised the need to investigate spatial distributions of the tissue types when regulated by different stimuli using finite element analyses.

The models were highly sensitivity to the length of the callus tissue, since it directly affected the boundary conditions. Its effects on model predictions were more discernible in models regulated by S3 rather than S1 and S2. The S3 models that were short (i.e. 1.5, 3.0 and 4.5 mm) inhibited healing. This finding would suggests that S3 may not be the best mechanoregulator for healing tissues in short lengths, which were normally found during early and late stages of healing (middle stage has the longest dimension) (Eliasson, Andersson et al. 2009). However, models regulated by S1 and S2 of all lengths successfully completed healing, with the predicted fibre re-organisation comparable to experimental findings. The predicted progression of the fibre re-organisation started on the outer layer near the tendon stump, which was in agreement with a histological study by Sasaki (Sasaki, Yamamoto et al. 2012). The complete results from their experimental study are briefly summarised here. Seven days after surgery, new collagen fibres grew extensively in the repair site in a random arrangement. Fourteen days after surgery, the collagen fibres began to form an axial arrangement. Near the tendon stump, this change progressed from the outer layer to the core region. On the other hand, in the middle of the repair site, it progressed from the core to the outer layer.

This study has shown that longer models completed healing faster than the shorter ones, for all stimuli regardless of loading magnitude. As shown in Figure 5.6b, the thresholds changes due to load increment reduced with length, which suggested transitions from one tissue to another were easier with length, hence causing faster healing. For instance, longer model of length 7.5 mm required lesser S1 strain of 8.29% for tissue type F0→F1 turnover, as compared to shorter model of length 1.5 mm which required higher S1 strain of 46.24%, when subjected to load of 1.0 N (Table 5.6a). The same pattern could be seen for higher loading magnitudes of other stimuli.

Also analysed in this study was the sensitivity of the models to loading magnitudes within physiological values. Models regulated by S3 were found more sensitive than those regulated by S1 and S2 (Fig. 5.10 and 5.11) where the longer models (6.0 and 7.5 mm) required more iterations to complete healing (Fig 5.13). This behaviour was not seen in S1 and S2 models despite the same change (increase with load) of the recalibrated thresholds (Fig. 5.6a) being observed. A previous study by Chen (Chen 2006) analysed the relation between uniaxial tensile stress and filament in a composite 3D model and showed that deviatoric strain (S3 in this study) was highly sensitive to loading directions. Their study showed that if load is applied perpendicular to the transversal radius that is shorter in length to the longitudinal radius (uniaxial tensile - this one is of our study), the deviatoric strain in the filament will monotonically increase. This hence further proved why higher tensile load delayed the healing process in S3 models, since the increment of deviatoric strain as a mechanical cue at tissue level (as a result of higher tensile load applied on the models) promoted formation of more disorganised fibre structure, which further inhibited maturation of the healing tendons.

The delayed healing predicted by S3 model in response to higher tensile loading contradicted findings of previous works, whereby physiological strain favours formation of soft tissues (Claes, Heigele et al. 1998; Schepull, Kvist et al. 2007; Eliasson, Andersson et al. 2009; Schepull and Aspenberg 2013). A study on plasticity of human Achilles tendon in response to cyclic strain (Arampatzis, Peper et al. 2010) demonstrated that exercises involving high tendon strain (mean, 4.72%) were more effective than those producing low tendon strain (mean, 2.97%) in triggering adaptive response. Stanish (Stanish, Rubinovich et al. 1986) suggested that eccentric exercises (which refers to straining exercise) prepare patients for return to functional, sports–related activities better than those that emphasise concentric muscle strengthening.

Underlying mechanobiological concepts that might elucidate why axial and principal strain (S1 and S2, respectively), were superior to deviatoric (S3), was somewhat discussed by Pauwel (Pauwels 1960) and Carter (Carter, Beaupre et al. 1998). In a compressible, elastic, isotropic material, hydrostatic stress causes a change in material volume, or volumetric strain, but no distortion. Conversely, octahedral shear stress (or call distortional stress) causes material deformation, or distortional strain, but no change in volume. They also mentioned that distortional stress and its resulting distortional strain always involves materials elongation in some direction, and therefore is associated with a tensile strain in that direction. The latter theory supports the finding that the axial and principal strains were better mechanoregulators than the deviatoric strain. Also due to the nature of tendons wherein fibres run parallel to tendon's long axis, mechanical signals that restore this structure both during regeneration and homeostasis are important. Studies by Barocas and Tranquillo (Barocas and Tranquillo 1997a; Barocas and Tranquillo 1997b) formulated a theory to study traction induced deformation and reorganisation of the extra-cellular matrix that accounts for contact guidance and the coupling of cell traction forces to the mechanical state of the matrix, and collagen alignment with the principal strain direction was assumed. They successfully predicted cellular alignment in mandril compacted tissue equivalents (Barocas, Girton et al. 1998). Since tendons are somewhat equivalent to the tissue (when it comes to fibre structure and cellular behaviour), their study further supported the finding that axial and principal strain are superior to deviatoric in regulating healing.

The similar spatial distributions predicted by axial and principal strain were possibly the result of simplified boundary conditions used in the FE analyses. Different patterns of fibre reorganisation may have been possible if the models were allowed to rotate due to torsion (if only torsion is also a loading condition during the healing process,

which is beyond the scope of this thesis). Note that the principal strain is an invariant, which is unaffected by changes to the coordinate systems (similar in the case of the deviatoric strain). The axial strain, however is highly dependent on coordinate systems, e.g. after deformation, which causes its value to change accordingly.

This study hypothesised that lower strain subjected to healing soft tissues would promote formation of more organised fibre structure than higher strain, within physiological loading magnitudes. Experimental studies have shown that optimum mechanical stretch is important to assist physiological cellular behaviours. For instance, a study of different coating materials on focal adhesion, comparing fibronectin and type I collagen, has shown that the latter induced physiological responses of low mechanical stretch which is advantageous for cellular mechanoresponse within physiological environment (Kim, Musson et al. 2016). This finding somewhat supported the aforementioned hypothesis on mechanoregulation of fibre re-organisation in healing tendons, wherein the resulted predictions by the developed mechano-adaptive models were comparable to experimental works.

Special attention was paid to the mathematical and computational simplicity of the models used in this study by introducing some assumptions. Firstly, it was assumed that the fibre remodelling for tissue maturation can be characterised by changing the fibre dispersion parameter of the GOH model, from high to low. The collagen fibre remodelling was not modelled explicitly since the required experimental data was not available. This challenge has been explained in Chapter 4 (Bajuri, Isaksson et al. 2016). Baaijens (Baaijens, Bouten et al. 2010) reviewed previous models used to simulate collagen remodelling.

An ideal model is thought to have a capability to alter its dimension according to its internal and external mechanical stimuli together with its respective bio-chemical factors.

This study, however has simplified this phenomenon using unchanging tissue dimensions for the whole of the healing process to ensure controllable and quantifiable simulation. Limited experimental information on the formation of external callus and its constituents is available, limiting the development of such models (i.e. callus growth simulation). Sasaki (Sasaki, Yamamoto et al. 2012) successfully mapped the collagen fibre arrangement during healing, and yet the study was lacking in information on external callus formation. Another more recent study by Tavares (Tavares, de Castro et al. 2014) had used magnetic resonance imaging (MRI) with perfusion for evaluating tissue and fibrous scarring in tendons. They managed to get high contrast images of scarring tissue; however, images of its progression during healing were not reported. In addition to the static dimension used, models created in this study had a rectangular shape, where the cross-sectional area (CSA) of the repair site was assumed to have the same magnitude as the tendon stump. Further information regarding the shape of the callus during healing is vital as it influences particularly the direction of the progression of the fibre re-organisation, as seen in a pilot analysis performed in our study.

In addition to collagen fibre which made up the solid phase of a tendon, another important element is water, which composed 70% of tendon's constituents (O'Brien 1997). Water content changes in healing tendons (Sharma and Maffulli 2006; Oakes 2008), and based on previous studies on bone healing mechanobiology (Carter, Beaupre et al. 1998; Isaksson 2012), water is speculated to impose biophysical stimulation (e.g. fluid flow and hydrostatic pressure) on healing tendons as well. It is thus imperative for future works to consider this component in the constitutive formulation. Khayyeri Poroelastic constitutive models, explicitly representing solid and one or more liquid phases, have been successfully used to describe this highly hydrated tissue. Implemented as part of a hyperelastic fibre-reinforced continuum poroviscoelasticity, the models were able to

describe both quasi-static and dynamic tests, as well as the direction of the fluid flow in the Achilles tendons of rats (Khayyeri, Gustafsson et al. 2015; Khayyeri, Longo et al. 2016). In future works, the GOH model used in our study, which focussed on the solid phase of the tissue, could be compared with the poro-viscoelastic model proposed by Khayyeri (Khayyeri, Gustafsson et al. 2015) in predicting healing tendons.

Also, an assumption has been made in terms of material properties used in this study, e.g. properties of healing tissue on day 3 (Eliasson, Andersson et al. 2009) was assumed to represent disorganised fibre. This thesis has so far been the first to categorise fibre structure in soft tissue healing, which is believed to be important to track progression of the tissue during regeneration. The idea however was lacking experimental data for model development and validation. Future works are therefore suggested to conduct proper procedures in which histological study with detailed-localised fibre structure, together with mechanical testing on the same sample, to measure its local mechanical properties. Hase (Hase, Minamikawa et al. 2016) suggested to observe tendon repair histological and mechanical behaviours of the same sample using second-harmonic-generation microscopy.

This study has assumed that no cells are involved in the healing process, hence also omitting the heterogeneity of cell density across the healing tissues (Sharma and Maffulli 2006; Nourissat, Berenbaum et al. 2015). Histological findings by Sasaki (Sasaki, Yamamoto et al. 2012) have shown that during early stage of healing, haematomic condition followed by random fibres production were found in the middle of the repair side at the core region. This was then shown to lead to transverse progression of the fibre re-organisations, from the core region to the outer layer (referring to the middle repair site). In order to simulate cell density and its mechanisms, a few factors need to be taken into consideration; sources of cells, especially fibroblasts, need to be addressed first, as figured by experimental works on bone healing.

Thresholds for the transition from one tissue type to another were calculated without being validated by experimental works, given that this study is the first exploration of the potential of developing mechanoregulation algorithm for healing tendons (hence has not reached the full way of thresholds establishment). We are also aware that the mechanosensation of the cells is most likely not changing during healing. It is not possible to be precise in selecting thresholds since there was no such data have been published. Nevertheless, this study has started by using the reported ranges of fibrous tissues formation, to guide the selection on the proposed thresholds. For instance, the formation of fibrous tissues have been experimentally measured to be either greater than 5% (Carter, Beaupre et al. 1998) or 15% (Claes and Heigele 1999) of principal strain. However, it is to be hoped that experiments on stem cells in accurately known mechanical environments (Thomas 2001) will allow the thresholds to be determined with more precision in the future (Lacroix and Prendergast 2002). Also a recent study which has used combinations of histomorphometric and numerical analyses to identify thresholds of strain intensity for bone tissue formation (Suzuki, Aoki et al. 2016), could also be considered. It is also important to note that the sensitivity of the thresholds values obtained was very high. If the significant digit was altered, for example by changing the limit for more-organised tissue to form from 0.07 to 0.08, the healing progression was inhibited.

This study analysed the sensitivity of the model to different lengths and loading magnitudes, but has yet to test different other factors, e.g. parameters in the constitutive GOH model. The parametric study performed in the previous chapter (Bajuri, Isaksson et al. 2016) has shown that parameters related to fibre are the most significant in capturing biomechanical behaviours of healing tendons, as compared to those related to non-collagenous matrix. However, this was only tested on specific timepoints of healing with no mechano-adaptation being involved. A pilot analysis to test how important the fibre

dispersion parameter, κ was in the mechano-adaptive environment has been performed. A clear difference, especially in spatial distributions, was observed whereby models with constant κ have less spatial definition than those who varied κ (signifying maturity). Future works are thus recommended to further such sensitivity analyses for better understand of the dynamics of the model and its formulation in predicting healing tendons.

Collagen fibre reorganisation was mechanistically modelled without incorporating its real deposition and realignment rates, rendering the modelled time an approximate, at best. Comparisons were made with the experimental results by identifying similar sequential fibre reorganisations instead of focussing on specific iteration numbers. The absence of physiology-based time dependency in the remodelling constitutes a limitation of the current formulation, as the time factor is indeed significant in optimising treatments for ruptured tendons (i.e. expediting the process of healing has long been a target, and a model that is unable to accurately predict temporal distribution is at a disadvantage).

The modelling of temporal distribution of healing tendons requires information such as rate of collagen production, migration rate of fibroblasts, production rate of extracellular matrix, and the rate of fibre re-alignment. Isaksson (Isaksson, van Donkelaar et al. 2008a) suggested the usage of coupled non-linear partial differentiation equations to simulate both cellular mechanisms (e.g. proliferation, migration, and production of extracellular matrix) and mechanical stimulation. This work used a new finite element formulation, wherein new elements were implemented in a finite element solver as a user-defined element. The migration rate and the rate of fibrous tissue formation used in the formulation were 40 μm/min (Sherley, Stadler et al. 1995; Friedl, Brocker et al. 1998) and 0.3 pg/cell h (Howard, Kucich et al. 1998), respectively. This work established the model's applicability by correctly predicting cell and tissue differentiations during normal fracture healing, with satisfactory temporal distributions of the respected cell and tissue. In

the case of the rate of fibre re-alignment, its formulation has been qualitatively suggested by Baaijens (Baaijens, Bouten et al. 2010). However, to the best of our knowledge, its implementation in finite element analyses has not, as of yet, been quantitatively addressed. We assumed that this requires the same approach reported by Isaksson (Isaksson, van Donkelaar et al. 2008a) through the use of subroutine in finite element solver incorporating partial differential equations to execute the new formulation. The method used in these works could be used to improve the temporal distribution modelled in this thesis.

In conclusion, a mechano-adaptive computational procedure to simulate fibre re-organisation in response to biophysical stimuli has been developed using a hyperelastic fibre-reinforced continuum model with distributed collagen fibre orientations. All parameters in the constitutive model were involved in the mechano-adaptive process. The models sensitivity was tested by attempting to simulate healing tendons in different lengths and subjected to different load magnitudes. The results show tissue formation patterns generally similar to those observed in experiments when regulated by principal and axial strain, but not by the deviatoric. Models regulated by deviatoric strain show delayed healing with the increase of tensile loads, and are relatively more sensitive to lengths; wherein these predictions were in contradiction to experimental findings. Overall, this study suggests further investigating each of the potential biophysical stimuli, given sufficient experimental information with relevant mathematical formulation, as different predictions of healing tendons were demonstrated.

6

Discussion and conclusions

The last chapter discusses the findings of the thesis in the context of past research and future prospects, and summarises its conclusions. In particular it provides the road to the development of further, more detailed models to clarify the relationship between mechanical simulation and biological response in tendon healing, and discusses the advantages and disadvantages of this approach. Finally it explores how knowledge of mechanical factors can potentially be used to enhance treatments for ruptured tendons, and the role that computational models can play in the development of those treatments.

6.1 Overview

In the first chapter, the need for further research in the field of tendon mechanobiology and its regulatory pathways was established. Tendon healing remains a costly, painful, and a disabling problem, with a lengthy healing process resulting in healed tissues with a reduced modulus. Over recent decades, the incidence of tendon rupture has increased due to aging of the population and more active lifestyles. Understanding the basic biology and the mechanoregulatory mechanisms of tendon regeneration will lead to faster, cheaper and more effective treatments.

The mechanoresponsive behaviour of tendons, in particular during healing has been extensively discussed in Chapter 2. Mechanical loading has a positive impact on a number of different mechanisms which potentially could address the problematic scar formation and slow process of healing. However, previous works have highlighted that care must be taken in the application of load to enhance tendon healing. A fine balance must be kept between under-stimulating and overloading the repairing tissue, since a complete removal of load is detrimental while large forces are also harmful. Optimal loading conditions hence need to be defined, and computer simulation, giving access to mechanical parameters not measurable in in vitro or in vivo experiments, offers the potential for achieving this.

Previous computational models simulating soft tissue biomechanics and mechanobiology have been developed for all scales – tissue, fibrillar and cellular, as described in Chapter 3. Both homogenous continuum models and models with explicit representation of tissue constituents have been used successfully to fit experimental data. Despite encouraging early results, the full range of computational mechanobiological modelling tools has not been applied to study tendons. The tools need to be developed in parallel with an increased availability of high quality longitudinal data from experimental

studies either directly in the clinic or using animal models. Such data is lacking for tendon adaptation, healing and degeneration and but is essential to enable validation of computational modelling frameworks to achieve clinical impact.

In Chapter 4, the ability of a constitutive model based on a hyperelastic fibre-reinforced continuum formulation introduced by Gasser-Ogden-Holzapfel (GOH) was tested to capture the biomechanical behaviour of healing tendons. The model has a combination of structural and phenomenological parameters. In particular its representation of distributed collagen orientations was used to signify the maturation of the healing tendons. Good fits to experimental tensile test data were obtained at all timepoints of healing. The sensitivity of the parameters in this model was analysed using a design of experiment (DOE) approach. DOE enabled a reliable sensitivity analysis to be performed with a small number of computational experiments. It was found that the GOH parameters that are related to collagen fibres are more important as compared to the parameter that represents the combination of the non-collagenous matrix neo-Hookean shear modulus and its volume fraction.

The GOH model was later used to develop a first mechano-adaptive model to investigate the role of mechanical factors in tissue differentiation during tendon healing (Chapter 5). The underlying hypothesis is that the magnitude of mechanical stimuli at a local tissue level influences the temporal and spatial tissue distributions which determine the biological repair process. Cells in the tissue are hypothesised to act as sensors, and to respond actively to mechanical stimuli by altering the local tissue microstructure, in particular changing the orientation of collagen fibres. To investigate this hypothesis, putative stimuli must be determined at numerous local positions within the healing tissue. A finite element model was used to represent the geometry of a healing tendon, and the tissue material parameters were updated iteratively. Using this platform several different

stimuli, loading magnitudes and model geometry were investigated to characterise their ability to represent qualitatively and quantitatively the time course of healing as documented by mechanical and histological analysis of animal healing models. Shortcomings of the algorithms were identified and strategies to overcome them were shaped.

6.2 Contributions and conclusions of the thesis

This thesis proposes a computational platform providing the first ever geometrically and temporally patterned mechanobiological model of healing tendons. This effort involved the selection and evaluation of a constitutive model, also applied for the first time to healing tendon, and the development of a coupled finite element analysis / Matlab code for an updatable, iterative representation of the healing tissue. The constitutive model in particular enabled the representation of the organisation of collagen fibres during tendon healing.

The constitutive model based on continuum fibre reinforced finite element model with distributed collagen fibres orientations introduced by Gasser-Ogden-Holzapfel, successfully captured the biomechanical behaviour (i.e. tensile strain) of healing tendons at specific time points – day 3, 7, 14 and 21 after transection – as well as of intact tendons. The implementation of this model in a finite element environment was shown, which allowed further assessment of stress and strain distribution across the healing tissue. This work further supported the use of the GOH fibre dispersion parameter κ, to represent tendon maturation via collagen fibre re-organisation in healing tendons, and showed that this parameter, as well as the fibre stiffness at low strain, $k_1^{'}$, and stiffening behaviour at large strain, $k_2^{'}$, are the most important GOH parameters for describing healing tendons mechanics under tensile load.

The mechano-adaptive FE model constructed based on the GOH constitutive formulation, satisfactorily predicted the fibre re-organisation pattern in healing tendons, with differences observed when the model was regulated by different biophysical stimuli, and subjected to different loading magnitudes as well as boundary conditions. Using axial and principal strain as stimuli predicted spatial and temporal patterns of tissue differentiation closer to experimental findings than deviatoric strain. These are preliminary findings that need to be confirmed by addressing the identified limitations of the modelling platform.

This finding of axial and principal strain to be the most promising regulators for healing tendons applies to collagen fibre re-organisation during the remodelling stage of healing. The cellular and other biological factors neglected in our model may be more important at earlier stages of healing. The poor performance of deviatoric strain may arise from the predominantly axial arrangement of fibres required in the healed tissue.

Future models may include a fluid phase into the formulation (i.e. poroelastic or poroviscoelastic formulation), since tendon derived fibroblasts have been shown to be responsive to fluid flow (Wall and Banes 2005). It is speculated that fluid flow may be most important during early healing stages, not captured in the work presented in this thesis.

Finally this thesis shows that computational models are an important tool in studies of tendon mechanobiology. The combined studies have demonstrated both the possibilities and limitations of computational models for tendon healing simulations. The studies presented in this thesis were compared extensively with a variety of experimental data, which led to future prospects of the development together with its validation. Such an approach will be discussed in the following sections.

6.3 Limitations

Mechanobiological computational models must always be developed in parallel with the availability of high quality experimental data describing the phenomena they attempt to predict. As previously discussed, such data are not widely available for healing tendons. Further, model development inevitably requires simplification to enable a particular research question to be addressed. The limitations of the work performed in this thesis are discussed in this section.

The mechanobiological analyses of healing tendons using computational modelling presented in this thesis, have made use of simplifications in terms of material characterisation and application of boundary conditions. Throughout this work, the maturation of healing tendons has been represented by the reduction of fibre dispersion, a particular parameter in the GOH constitutive model. This resulted in the transformation of the material from isotropic to anisotropic during healing (Chapter 4). Fibre dispersion is a measurable parameter, however, there are no published reports of its value during tendon healing and its estimation from published images of healing tissue is unreliable and limited to a few cases.

Different tissue types based on collagen fibre structure were specified in the presented computational platform, to capture progression of the healing tissue with respect to fibre re-organisation, i.e. F0-disorganised; F1-partially organised; F2-moderately organised; F3- well-organised. The healing tissue will of course vary continuously both spatially and temporally, while these discrete tissue types with material properties are not well established. Despite this the spatial and temporal sequence of tissue differentiation was reproduced (Chapter 5).

All studies of tissue constitutive formulation and differentiation presented so far (Chapter 4 and 5) have been conducted at the tissue level. An assumption is that one tissue

type can transforms into another tissue type, merely based on collagen fibre re-organisation. Is this description sufficient? Experimental findings on tendon remodelling demonstrate other factors involved during the process, i.e. angiogenesis, water content changes, cell proliferation etc., all of which may also be dependent on mechanical stimulation. Previous tissue level models of bone healing (Isaksson 2012) included these biological factors in their continuum formulation, given that sufficient experimental data were available for both model development and their corresponding validation. It is thought that the future advancement of tendon healing modelling would to some extent be motivated by approaches used to model bone healing. However, adding complexity to a model is not always better. The necessary level of complexity will depend on the research question, and one should keep a model as simple as possible, as long as it can answer the particular research question, within the boundaries of the current knowledge.

Equally important when performing finite element studies are the descriptions of the boundary conditions, including the load application. The modelling presented here used simplified boundary conditions and load application which may not adequately represent the in vivo experiments. However, as discussed earlier loading in animals is very difficult to control or measure.

Several authors have discussed the use of computer models to evaluate mechanical stimuli at a macroscopic (homogenised) continuum level. With our hypothesis that the tendon cells are the sensors that react to local stimuli, it is not clear whether the continuum approach is completely valid (van der Meulen and Huiskes 2002). The GOH model used in this thesis, despite having structural parameters, i.e. fibre dispersion, κ and preferred direction, γ, the collagen fibre stiffness parameters k_1' and k_2', does not explicitly represent the microstructure of the tissue in which the cells are embedded. With the rapid development of measuring and imaging tools both for material properties and histology,

such as second-harmonic generation microscopy (Hase, Minamikawa et al. 2016), more reliable and realistic constitutive formulation and geometry representation will be possible. Therefore, in the future, microscopic scale models (homogenised at a micro-scale instead of macro-scale) that can be used to investigate the actual stimuli acting at a fibrillar level will probably become important. This development is already evident in the use of multi-level modelling where both a continuum and a micro-scale modelling approach are combined to simulate healthy tendons (Young, Gardiner et al. 2016). However, this has not yet been applied to healing tendons.

Despite the limitations mentioned above, which in many cases are also concerns in both in vivo and in vitro experimental studies, numerical models have been shown to be very useful in examining possible mechano-regulatory pathways. This has been further established by the work presented in this thesis. In the future, such modelling techniques might be used to study more problematic cases of tendon healing, and also to identify new future research questions.

6.4 Future directions

Surgical tendon repair methods suffer from high rates of failure. For Achilles tendon, the rate of re-rupture was reported to be 1.7 - 5.6%, occurring between 4 and 13 weeks after treatment (Hanada, Takahashi et al. 2011). Contributing factors included skin scarring and shortening, adhesion between the subcutaneous scar and the suture of the paratenon, as well as post-operative immobilisation. For rotator cuff injury, the picture is worse with rates of re-tear following surgical treatment of up to 94% (Bishop, Klepps et al. 2006). Several factors associated with failure of surgical repair have been reported, including advanced patient age, large size of tear, severe muscle atrophy and fatty infiltration, systemic diseases, and smoking (Montgomery, Petrigliano et al. 2012).

Further, tendon has low cellularity (as opposed to epithelial tissue which has high cellularity), which explains the low turnover and poor self-healing capacity of the tissue (Bi, Ehirchiou et al. 2007; Tan, Lui et al. 2013). This is exacerbated by a poor blood supply, together with poor angiogenesis during the healing process (Petersen, Hohmann et al. 2002; Benjamin, Kaiser et al. 2008). Another factor is the microenvironment after injury, which may induce erroneous differentiation of the resident stem cells, and cause pathological tendon ossification (Lui and Chan 2011; Rui, Lui et al. 2011; Lui 2013).

The computational modelling platform developed in this thesis provides a simulation for the mechanobiology of healing tendons. This platform could be extended to study tissue regeneration based on collagen fibre re-organisation in tissue engineering constructs, which are stimulated mechanically. The platform will allow controlled variations of mechanical stimulation and boundary setting. Hence, it is a good early computational model to obtain quantitative data and relationships between certain parameters. The platform currently does not model the spatial or temporal distribution of several biological factors, but these could be introduced. For example collagen density, cell distributions and rate of collagen synthesis, growth factors and cytokines are all simulated in other published mechanobiological models.

The computational platform developed in this thesis could explore how exogenous mechanical or biological factors from a novel regenerative therapy might accelerate healing. The effect of different tendon geometries and types could be investigated to propose anatomical site specific therapies for the different problems of rotator cuff and Achilles tendon injury.

As discussed the parallel development of computational models with descriptive data of the biological processes is required. However, some scientists have argued that validation of numerical models of natural systems is impossible (Oreskes, Shrader-

Frechette et al. 1994), and correctness of model prediction cannot be proven, only disproven (Popper 1959). Therefore, tolerance levels must be defined and hypotheses tested in terms of whether sufficient validation can be achieved. According to Anderson (Anderson, Ellis et al. 2007), corroboration of a computational model is achieved when enough evidence is generated and credibility established that a computer model yields results with sufficient accuracy for its intended use. That begs the question of what is sufficient accuracy. There is no universal answer to that question, but for some models achieving predictions within the experimental variability might be considered sufficient (e.g. Chapter 4, Fig. 4.5).

In general, works conducted in this thesis produced results that were comparable to experimental findings. The few experimental data that are available have been used for model development, but no additional data was available for model validation. Future works in validating and surely improving the model development could be achieved if the aspects discussed were properly addressed.

Computational models have been shown to be useful for research in bone and wound healing, whereby numerical methods have been used to formulate and simulate complex biological problems (Peña, Calvo et al. 2006). In vivo experiments are costly, difficult to perform, with limited control over important variables such as loading conditions and geometry, and data are only available at a limited number of discrete time points during the course of healing. Computational models can provide continuous evolution in time through interpolations. Parametric investigations, which are almost impossible to examine experimentally, can be performed systemically with computational models (Prendergast, Huiskes et al. 1997). The work presented in this thesis demonstrates these advantages.

The goal of mechanobiology applied to tendon healing is to search for relationships between mechanical factors and cellular and tissue response. However, to establish the interdependence of biophysical stimulation and tendon repair and remodelling at the material and structural level, experiments must be carefully designed (van der Meulen and Huiskes 2002). These studies include a) appropriate animal models to investigate the cellular and tissue responses under different forms of mechanical stimulation; b) in vitro cells and tissue culture studies with well-controlled biophysical stimuli to eliminate other confounding factors at the systematic level and c) computational models to investigate mechanobiological relationships and possible signalling pathways (van der Meulen and Huiskes 2002).

Most experimental and computational models of tendon investigate tissue differentiation under a known tensile load, which is assumed to be the main stimulus, with much less information about loading during the repair process. Given that tendon healing modelling is relatively new compared to modelling of other skeletal tissues, both qualitative and quantitative conclusions are still far from final. It is even more pronounce given apparent differences between individual patients and animal species. This is also the case for in vivo and in vitro experimental models. In particular, identification and inclusion of the effects of patient variability and patient ageing will become important aspects. As highlighted earlier, the burden of tendon ruptures is increasing, which thus makes efforts to quantify the response and variability of physiological parameters between individuals and animal species an imperative task for the future. Future research in tendon biomechanics and mechanobiology will bring more complex and realistic computer simulations (on top of hypothesis driven model development) and will reduce animal experimentation and clinical trials, with related economic benefits. With the progress made in this field in recent years and the work conducted within this thesis, efforts are now starting to show

that computational modelling can be of help to better understand tendon mechanobiology, similar to work conducted on other skeletal tissues.

In the near future, the work presented in this thesis could be extended in three major directions. Firstly, models for cell anabolic and catabolic processes, especially of collagen, are required, predicting quantities and orientation of the fibrils secreted, as well as quantities of matrix proteinases. Secondly, micromechanical models of the cell local environment are required, coupled with the local microstructure of the extracellular matrix, which both is capable of predicting local micromechanical environment (e.g. shear stress) in an adaptive manner. Thirdly micromechanical models need to be linked to macroscopic deformation, requiring detailed knowledge of tendon hierarchical organisation and macroscopic geometry. This could be achieved using a multi-scale finite element representation.

In the far future, it will be important to focus research on the integration of simulations, experiments and theoretical aspects (van der Meulen and Huiskes 2002). Not only should there be greater interaction between experimental studies and computational modelling, but experiments should ultimately be designed and carried out with the associated computational investigation in mind, in order to improve the value of numerical modelling. Use of computational techniques for parametric examination of factors that are difficult or impossible to examine experimentally will contribute to the advance of biomechanics and mechanobiology of tendons, as indicated in this thesis and by others (Prendergast 1997; Prendergast, Huiskes et al. 1997; Smith, Rubenson et al. 2013; Thompson 2013)

Eventually, once the influence of mechanical stimulation on transcription factors, signalling pathways and genomic elements have been elucidated, it might be possible to eliminate today's reliability on mechanical forces for stimulation and to induce these

signals by other means. This would result in simpler and more efficient rupture treatment and prevention. Perhaps, such technology could be used to restore collagen fibre structure in tendons systemically. Answers to questions such as these would have direct bearing on many of the clinical problems that now confront the orthopaedic community.

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