General introduction, thesis aim and outline

ANTERIOR CRUCIATE LIGAMENT RUPTURE AND TREATMENT

ACL rupture is a common sports-related injury potentially causing instability of the knee joint. In the general population, annual incidence rates reach up to 5 - 8 per 10.000 persons[1, 2]. On the contrary, incidence rates reported for professional athletes are substantial higher: 8 to 52 per 10.000 per persons per year in various populations including Sweden, Norway, Denmark, The United States of America, Australia and Germany[1]. However, the exact incidence in The Netherlands is unknown. ACL injuries are most frequently observed in pivoting sports, such as down-hill skiing, soccer, handball and basketball[3]. Women are at 2 to 8 times greater risk as men of suffering this injury[4, 5].
Currently, the treatment options are either a conservative regime with exercise therapy or a surgical reconstruction of the injured ACL. The Dutch ACL guidelines recommend surgical reconstruction only when knee instability exists. Otherwise, a conservative treatment is indicated[6]. When despite adequate conservative therapy complaints of instability remain, one might consider operative treatment too. Other factors that contribute to the final treatment decision are additional injuries and patient's requirements in terms of activity levels and participation in pivoting sports[6, 7]. The number of ACL reconstruction procedures performed globally and in The Netherlands is increasing[8, 9]. The estimated number of ACL reconstructions in The Netherlands in 2003 was 3.000[9], whereas today's estimations reach up to 7.000 reconstructions annually[10].
An important aspect of the ACL reconstruction procedure is the graft choice. Today, several graft options exist, including autografts, allografts and synthetic grafts. Because of unlimited access and no donor-site morbidity, synthetic grafts were popular in the past. However, these grafts presented serious drawbacks such as immunological responses, recurrent instability and knee osteoarthritis[11]. Therefore, artificial grafts are hardly used in current clinical practice[12]. There are various allografts available for reconstruction purposes, such as tibialis posterior tendon, tibialis anterior tendon, Achilles tendon, peroneus longus tendon and bone-patellar tendon-bone (BPTB). A potential disadvantage of the use of allografts is the risk of infection, graft rejection and graft elongation. These disadvantages are less likely to occur in autografts. Autografts are therefore the most preferred graft for ACL reconstruction procedures. The most commonly used autografts are the hamstring tendons and bone-patellar tendon-bone (BPTB)[13]. As BPTB grafts are associated with donor-site morbidity in 80% of the patients[14] and patellar tendon rupture occurs in 0.24%[15], the hamstring tendon autografts are the graft of choice to replace injured ligaments in the Netherlands as well as globally. Orthopaedic surgeons tend to harvest two hamstring tendons and subsequently fold them to create the typical 4-stranded graft. This ensures that an optimal graft size is obtained and so that optimal biomechanical function is reached[16].

LIGAMENTS AND TENDONS

Anatomy

The anterior cruciate ligament (ACL) is a ligament that courses from the femur to the tibia. More precisely, the ACL arises from the posteromedial side of the lateral femur condyl and attaches on the anteromedial side of the tibia plateau (Figure 1). The ACL is comprised of two bundles named for their insertion sites on the tibia plateau: anteromedial (AM) bundle and posterolateral (PL) bundle[17]. Its main function is considered the primary restraint to anterior displacement of the tibia and to provide rotational stability[18].

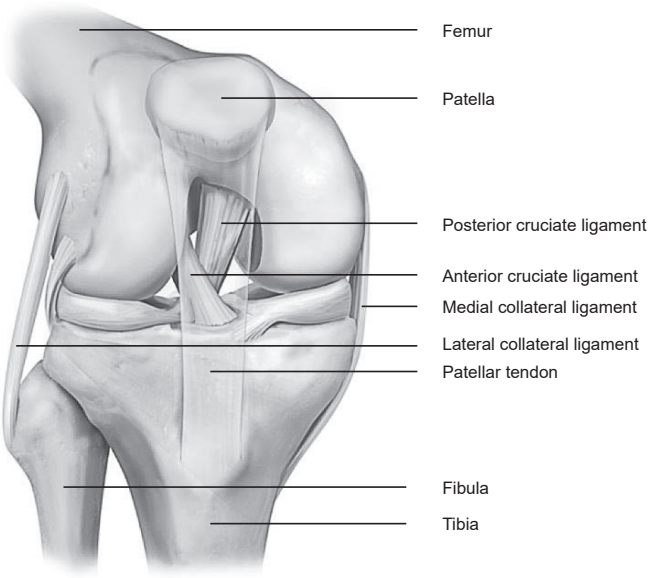

Figure 1: Anatomical representation of the right knee (modified from Kennedy et al.[19]).

The two tendons that are harvested for reconstructive purposes are the semitendinosus and gracilis tendons. The semitendinosus is located in the postero-medial side of the thigh and has its origo at the inferior-medial aspect of the ischial tuberosity. The proximal tendon shares a tendon with the biceps femoris. The long distal tendon, which is harvested for reconstruction of the ACL, starts caudal from the mid-thigh.

The gracilis tendon has it origo at the ramus inferior ossis pubis and descends along the medial thigh. From an anatomical and functional perspective, the m. gracilis is considered to be an adductor of the leg. The tendons of the semitendinosus, gracilis and sartorius eventually conjoin to form the pes anserinus. The pes then turns around the medial aspect of the tibia and inserts at the tuberositas tibiae.

It should be noted that in the light of autografts for ACL reconstructions, the m. semitendinosus and m. gracilis are often referred to as hamstring tendons.

Structure

Tendons and ligaments are hierarchically organised. The main structural component is collagen, which is a triple helix. The assembly of five collagen molecules is termed a microfibril. These microfibrils are arranged into larger longitudinal bundles. Depending on their size, these bundles are called subfibrils, fibrils and fascicles (Figure 2). Each fascicle is separated by a layer of loose connective tissue that is known as the endotenon. A group of fascicles form the entire tendon, which is enclosed by the epitenon: a connective tissue-sheath containing the vascular, lymphatic and nerve supply. The ligamentous equivalent for endotenon is endoligament, whereas epitenon is referred to as epiligament. In general, the collagen fibers are organised in the direction of the applied force. As forces in tendons are applied in a uniaxial direction, a parallel alignment of the collagen fibrils is found in tendons. However, collagen fibrils are not as uniformly orientated in ligaments because forces are applied in more than one direction.

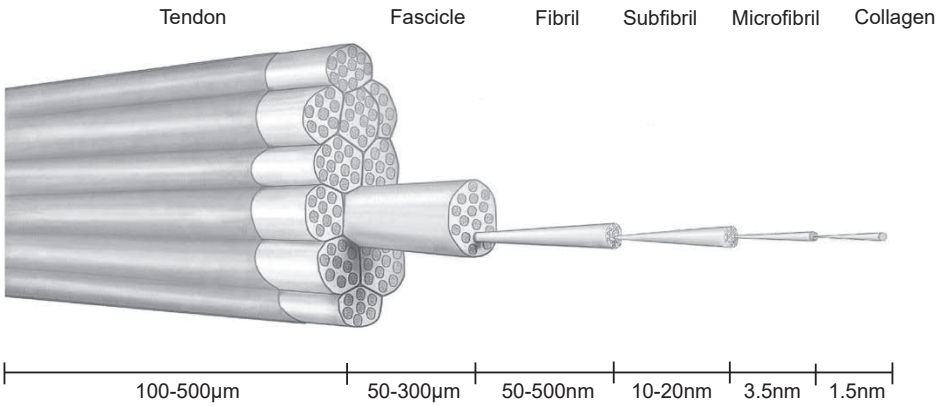

Figure 2: Schematic image of the hierarchical structure of tendons and ligaments (modified from Encyclopedia Britannica[20]).

Composition

The extracellular matrix (ECM) of tendons and ligaments is approximately composed of 65-80% collagen (dry weight)[21-23]. Collagen type I is with 95% of the total collagen the predominant collagen in both ligaments and tendons. Additionally, at least 28 more collagen types are found in minimal concentrations[24, 25]. Collagens contribute to the structural framework in tendons and ligaments as they form both intramolecular and intermolecular covalent cross-links. This stabilises the ECM and determines its tensile strength. Forms of cross-linking that are generally found in tendons and ligaments are the hydroxylysine aldehyde derived and the lysine aldehyde derived cross-links[26]. These are established after enzymatic modifications[27]. Another mechanism of cross-linking is via non-enzymatic modifications using glucose, with pentosidine as a well-identified end product[28].

Although collagen fibrils are the main component in the ECM of tendons and ligaments, several other non-collagenous constituents also contribute to its overall function. Proteoglycans, a special class of glycoproteins, represent 3% of the dry weight in tendons and ligaments[29, 30]. These proteoglycans contain glycosaminoglycan (GAG) subunits that, due to their high concentration of negative charge, generate an osmotic pressure by attracting water. The water content of the matrix is about 70% of the wet weight of the ECM. This leads to lubrication and spacing allowing fibers to glide over each[31].
Highly specialized fibroblasts are sparsely present in the ECM, but represent the main cell type in tendons and ligaments comprising 90-95% of the cell population[32, 33]. These fibroblasts in tendons are referred to as tenocytes and in ligaments as ligamentocytes. These cells are involved in the degradation and synthesis of ECM components.

TISSUE HEALING AND INFLAMMATION

It has been reported that hamstring tendons harvested for ACL reconstruction are able to regenerate after surgical resection[34]. These regenerated tendons clinically appear as a well-defined fibrous band that could be palpated on the posteromedial aspect of the popliteal fossa[35]. Macroscopically, regenerated tendons have the same colour and glossiness as those of normal hamstring tendons[35, 36]. In addition, several studies microscopically examined the regenerated tissue. No significant differences were found in terms of collagen type, fiber structure, cellularity, vascularity and amount of GAGs when comparing the regenerated tendon with native tendon[35-38]. This illustrates the remarkable extent of tendon healing following harvesting procedures.
Tissue healing is a complex and multistage process, involving the recruitment of various cells. These cells typically produce their own cytokines or growth factors contributing to the process of tissue healing. Tissue healing can be subdivided in four stages[39]:

1. Haemostasis: the blood clotting system is activated in the first minutes to hours after (iatrogenic) injury. More specifically, thrombocytes and platelets aggregate in a fibrin network[40]. Additionally, these platelets release cytokines and growth factors to attract other cells.
2. Inflammation: during this phase inflammatory cells are recruited to remove dead cells, bacteria and other pathogens. Together with macrophages, mast cells and T-lymphocytes are attracted and subsequently secrete multiple factors to influence the process of tissue healing[41]. This process typically takes a few days to a few weeks.
3. Proliferation: following the inflammatory phase, cells will start to proliferate and synthesize structural and fibril-associated components of the extracellular matrix. This step can take a few days up to weeks after injury.

4. Remodelling: in this final stage, the new tissue will be rearranged into normal tissue structure. In particular the orientation of collagen fibers is reorganised along the tension lines. Furthermore, superfluous cells will undergo apoptosis during this phase. In general, this phase might take weeks to months following tissue injury.

Macrophages are a major component of the mononuclear phagocyte system and are key role players in the inflammatory phase of the process of tissue healing[42]. These specialized cells of the immune system are derived from monocytes. Depending on the microenvironmental cues, macrophages are able to obtain a whole spectrum of different phenotypes with distinct functional and phenotypical characteristics[43, 44]. The tissue-remodelling process is orchestrated by these macrophages, but more specifically by their produced cytokines and chemokines[41].

Pro-inflammatory macrophages, or M1, represent one end of the spectrum. Their main function is to debride affected sites by phagocytosis of pathogens, foreign materials and damaged cells[45]. Also, pro-inflammatory macrophages are responsible for the production of numerous pro-inflammatory cytokines including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α. Conversely, on the other end of the spectrum, anti-inflammatory macrophages, or M2, are found. They are involved in tissue repair and healing processes by the secretion of anti-inflammatory cytokines, such as IL-4, IL-10 and transforming growth factor (TGF)-β[43, 44]. These cytokines, interleukines and growth factors are known to activate different pathways;

Apoptosis Inflammatory factors are known to stimulate the production of reactive oxygen species, resulting in the production of caspases[46, 47]. Caspases are known to induce apoptotic cell death[23]. A decrease in cell numbers directly compromises maintenance and repair of the ECM, as cells are responsible for the production of ECM components.

Fibrosis Other inflammatory factors contribute to an upregulation of TGF-β leading to an increased production of collagens and proteoglycans[48-51]. Ultimately, this might lead to fibrosis. In addition, TGF- β induces the synthesis of tissue inhibitors of metalloproteinases (TIMPs), preventing the degradation of matrix components[52].

ECM degradation The production of prostaglandin E_2 is induced following exposure to inflammatory cytokines and leads to an upregulated production of metallaproteinases (MMPs)[53-55]. These proteins are known to enhance the degradation of ECM components. Taken together, the inflammatory response is a complex combination of pro- and anti-inflammatory factors that needs to be tightly regulated. An imbalance between the pro- and anti-inflammatory response leaves the inflammation unchecked resulting in either too much matrix degradation or too much fibrotic tissue.

AIMS AND OUTLINE OF THIS THESIS

Anterior cruciate ligament (ACL) reconstruction has become standard orthopaedic practice worldwide and often requires harvest of the hamstring tendons. However, the harvest of functional and healthy tissue might lead to donor-site morbidity and functional deficits. In 1992, Cross et al. were the first ones to describe the remarkable feature of these tendons to regenerate following harvesting procedures, potentially solving the post-harvest morbidity[34]. Therefore, the general aim of this thesis is to improve the outcome after hamstring tendon harvesting through a better understanding of tendon regeneration.

In **Chapter 2** we conduct a systematic review to summarize the available literature about hamstring tendon regeneration following harvesting procedures.

Regeneration of the hamstring tendons has been associated with various clinical symptoms, such as pain in the posterior thigh, cramping and muscle weakness[56]. These symptoms might be explained by failure of the regeneration process or altered morphological properties of the regenerated tendons. **Chapter 3** describes the process of hamstring tendon regeneration at one- and two-years follow-up after ACL reconstruction entailing the hamstring tendons using magnetic resonance imaging. More specifically, it reports regeneration rates, changes in cross-sectional areas and tendon lengths.

Considering the clinical symptoms, it might be interesting to preoperatively identify patients that are likely to lack a regenerative capacity of the hamstring tendons. Knowledge about modulators for hamstring tendon regeneration might alter the graft choice. Therefore, **chapter 4** identifies predictive factors for hamstring tendon regeneration. In addition, patient-reported outcome measurements between patients with and without hamstring tendon regeneration are reported.

Inflammation is a well-known factor that contributes to tissue repair. However, a better understanding of the effects of inflammatory factors on the production of extracellular matrix components is required to direct the inflammatory process and to improve tendon regeneration. Currently, it remains unclear how polymorphisms within genes encoding inflammatory proteins such as *interleukin (IL)1B* and *IL6* affect the production of structural and fibril-associated components of the extracellular matrix. **Chapter 5** focuses on the effect of polymorphisms within genes encoding for two inflammatory factors (*IL1B* and *IL6)* on gene expression levels of collagens and proteoglycans in fibroblasts with an increased or decreased injury risk.

Immune cells, in particular macrophages, are the key role players in inflammation and are known to produce inflammatory factors such as IL-1β and IL-6. The production of these proteins is known to be stimulated following activation of a specific signaling pathway. **Chapter 6** describes the effects of specific inhibition of this inflammatory signaling pathway on macrophage phenotypes.

Finally, **chapter 7** summarizes and discusses the main findings and limitations of the studies described in this thesis. In addition, it combines the knowledge of the studies to discuss potential directions for future research in order to improve the outcome following hamstring tendon harvesting procedures.

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