

PRE-OPERATIVE DETERMINATION OF THE OPTIMUM GRAFT LENGTH FOR ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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Declaration of original work

I, **Reinette Ebersohn**, hereby declare that the dissertation entitled,

Pre-operative determination of the optimum graft length for anterior cruciate ligament reconstruction,

is my original work and has not been submitted to any other tertiary institute for degree or award purposes other than the University of Pretoria for the degree in M.Sc. Anatomy.



R Ebersohn

31-10-2014

Date

Foreword and acknowledgements

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Summary

Purpose:

Accurate knowledge of the anatomy of the anterior cruciate ligament (ACL) is crucial for successful ACL reconstruction. Incorrect graft lengths and/or tunnel misplacement have to be avoided. If the graft length is incorrect, the patient could risk knee instability, loss of range of motion or failure of graft fixation. The success of ACL reconstruction will be enhanced if the correct length of the graft ligament required, can be predicted in advance. Magnetic resonance imaging (MRI) is currently used for the evaluation of ACL injuries, but is not available to all patients. Apart from examining the morphological properties of the ACL at macroscopic and microscopic levels, this study aimed to determine whether independent factors of an individual can be used to predict native ACL length which could assist in pre-operative planning.

Methods:

Ninety-one adult cadavers were studied. The patellar ligament (PL) length, ACL length, ACL width and the maximum femoral epicondylar width (FECW) were measured. For the radiographic component, 52 patients were sourced to evaluate and compare ACL length, PL length and FECW, measured on both MRI and radiograph. Fresh ligaments were harvested (18 ACLs and 10 PLs) to evaluate the histological composition of the ACL and PL.

Results:

The morphology of the ACL and PL was determined. The morphology of the ligaments compared well to the descriptions in previous literature. The ligaments proved to be compatible at histological level. The results revealed that FECW was the most reliable predictor of ACL length. Linear regression formulas were developed in order to determine ACL length by measuring maximum FECW. It was also determined that either an MRI or radiograph can be used to assist in pre-operative planning.

Conclusion:

ACL and PL morphology compared well with the descriptions found in previous studies. It was also found that, contrary to previous studies, the maximum FECW is a more reliable predictor of ACL length than the height of the patient. The results also showed that both radiographs and MRI scans can be used to determine pre-operative ACL length. These results could improve the pre-operative planning of ACL reconstruction and minimise the occurrence of graft mismatch.

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List of abbreviations

ACL	Anterior cruciate ligament
AP	Anterior to posterior
BTB	Bone-patellar tendon-bone
ddH₂O	Double distilled water
FA	Formaldehyde
FECW	Femoral epicondylar width
FH	Feet to head
FOV	Field of view
GA	Gluteraldehyde
H&E	Haematoxylin and eosin
HS	Hamstring tendon
M	Molar
MCL	Medial collateral ligament
MRI	Magnetic resonance imaging
PCL	Posterior cruciate ligament
PL	Patellar ligament
RL	Right to left
SD	Standard deviation

Chapter 1: Introduction

Anterior cruciate ligament (ACL) injuries are very common^{1,2}, especially in sport activities that put high demand on knees, such as football.³ The knee is a low-placed, mobile and weight-bearing joint that functions as a fulcrum between the femur and tibia.^{4,5} The stability of the knee joint depends on the ligaments and muscles surrounding it. The mobility of this joint makes it prone to injuries, especially during rotational movements.⁴ The ACL is particularly at risk as it prevents the posterior displacement of the distal end of the femur on the proximal end of the tibia. However, during strong rotational movements of the knee joint the ACL can tear completely or partially.⁵⁻⁹ ACL tears are common sports injuries that could be career-limiting to men and women participating in sports. In cases where the ACL tears, reconstructive surgery is needed to re-establish the anteroposterior and rotational stability of the knee.¹⁰ Although different treatment options are available, including non-operative interventions, the best way of ensuring future knee stability is ACL reconstruction.¹⁰ The preferred graft used for ACL reconstruction is the bone-patellar tendon-bone (BTB) graft. For this graft the middle third of the patellar ligament (PL) is harvested along the long axis.^{7,11-14} It is therefore important to have a sound knowledge of the morphological characteristics of the PL and the ACL, as well as the relationship between the two, in order to obtain the most successful graft for reconstruction. Ligament grafts used in ACL reconstruction need to be the correct length for proper functioning. If the graft length is incorrect, the patient could risk knee instability, loss of range of motion, failure of graft fixation or osteoarthritis in the knee joint.^{2,8,11,15} Pre-determined length of the native ACL could assist with pre-operative planning of correct graft length which would make the ACL reconstruction easier, more time-efficient and also decrease possible post-operative complications. Magnetic resonance imaging (MRI) is currently used for the evaluation of ACL injuries and is considered to be the imaging modality of choice. Unfortunately, it is not readily available to all patients. It would be beneficial if another method, which is freely available, could assist in the pre-operative planning for ACL reconstruction.

Chapter 2: Literature review

2.1 Knee joint

The most composite joint in the body is the knee joint.¹⁰ It is classified as a synovial hinge joint^{4,10} and is the largest and the most superficial joint in the body.⁴ The hinge movements of this joint are combined with rolling, rotation and gliding around the vertical axis.⁴ The knee joint is complex requiring three translation and three rotation movements to enable a worthy description of the motions of this joint.¹⁰ The structural stability of the knee depends on the strength and action of the surrounding muscles and the ligaments connecting the tibia and femur. These structures are therefore particularly susceptible to injuries.^{4,10,16} The disruption of the ACL, for instance, will cause extensive disability for it may alter normal locomotion.¹⁰

2.2 Ligaments of the knee joint

As previously mentioned, the main function of the ligaments of the knee is to stabilise the knee joint. Ligaments are the static stabilisers of the knee. They control the normal kinematics and prevent the displacement and rotation of the knee joint that may damage the articular surfaces.¹⁰ The lateral compartment of the knee joint is more mobile than the medial compartment, which is the area where the ACL attaches.⁶ The ligaments are mainly composed of Type I collagen in a proteoglycan matrix. Varying amounts of reticulin and elastic fibres are present, as well as nerves and cellular and vascular elements. The collagen fibres are arranged in parallel bundles allowing it to withstand tensile loads.^{10,17,18} The ACL, which is the ligament of interest in this study, is an intra-capsular ligament of the knee joint.

2.3 Anatomy of the anterior cruciate ligament

The ACL is one of the two intra-articular ligaments of the knee joint, the other being the posterior cruciate ligament (PCL). The ACL originates from the posterior portion of the medial wall of the lateral femoral condyle (Fig. 2.1 a, b). The ACL courses anteriorly and medially through the knee joint to insert on the tibial inter-articular area. The insertion site is anterolateral to the medial tibial intercondylar eminence on the anterior intercondylar area of the tibia (Fig. 2.2 a, b).^{4,5,7-10,16,19-22} The ACL turns in a lateral spiral as it moves through the knee joint and rotates $\pm 90^\circ$ as it approaches the tibia. This twist is due to the placement of the bony attachments of the ligament. The attachment site on the femur is primarily oriented in the longitudinal axis, while that of the tibia lies within the anteroposterior axis.^{5,9}

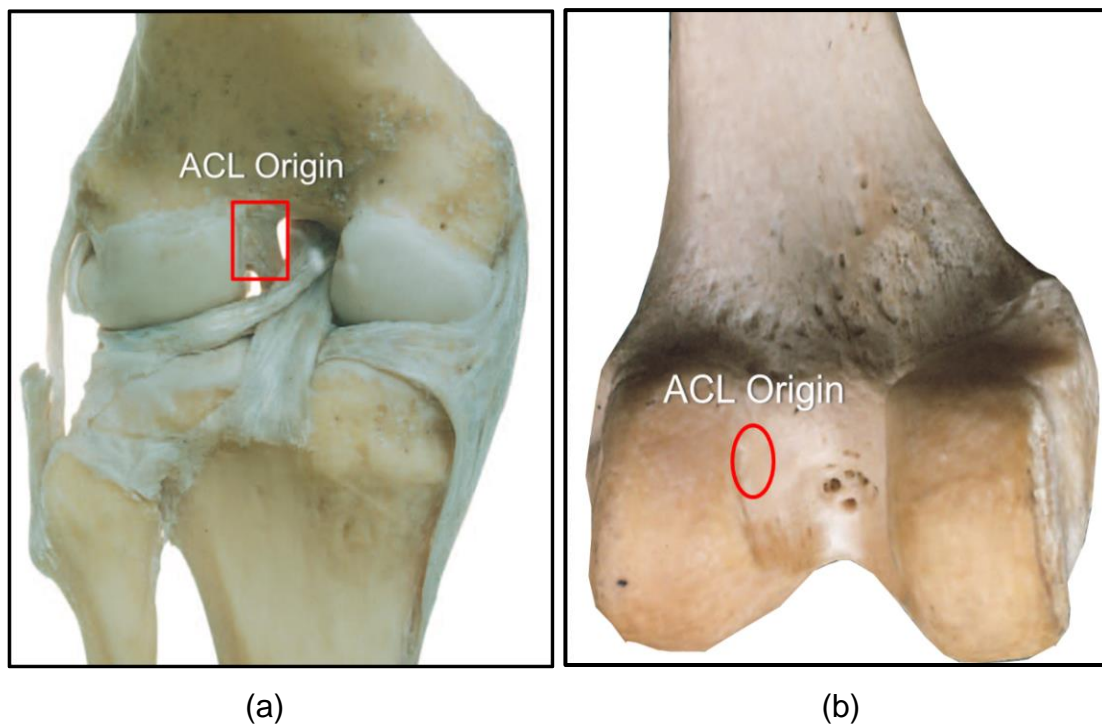


Fig. 2.1 a, b: Posterior view of the knee joint indicating the origin of the ACL.²³

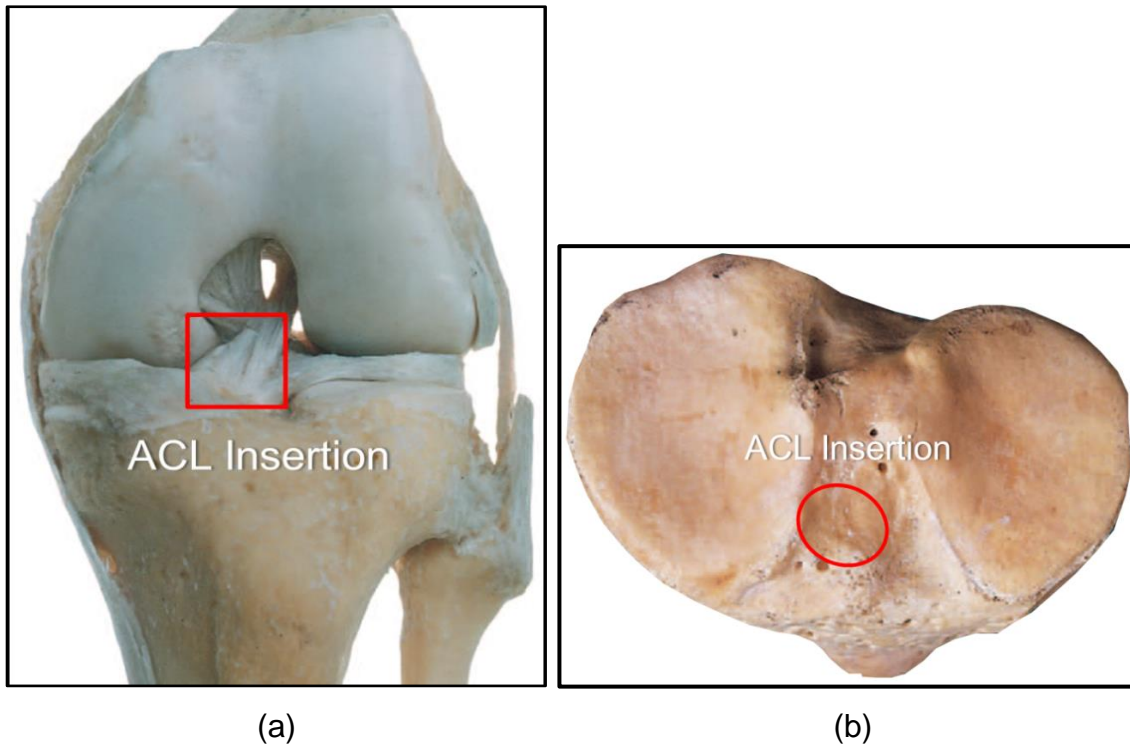


Fig. 2.2 a, b: Insertion of the ACL from an anterior (a) and superior (b) view²³

The ACL has a poor blood supply.⁴ The main blood supply of the ACL is from the middle genicular artery, which is a branch from the popliteal artery.^{10,16,20,21,24} Vessels from the middle genicular artery supply the proximal region of the ACL, while the distal region is supplied by the lateral and medial inferior genicular artery branches.^{5,7,8,16,22,25} The genicular artery gives off ligamentous branches at the dorsal surface of the ACL. These branches form a network to supply the ACL (Fig. 2.3, 2.4).^{7,10} Both the proximal and distal vessels support a synovial plexus. Small vessels run from this plexus into the ACL and align parallel to the collagen bundles. In the insertion regions there has been found to be avascular areas.^{5,22,24,25} These avascular areas could be the reason for the poor healing abilities associated with ACL injuries.



Fig. 2.3: Blood supply of the ACL from an anterior view.²⁴

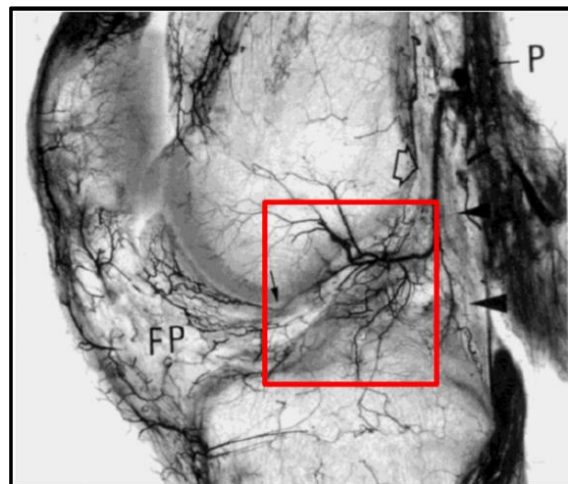


Fig. 2.4: Blood supply of the ACL from a lateral view.²⁴

The nerve supply of the ACL consists of the posterior articular nerve branches from the tibial nerve.^{7,10,16,20} The nerve supply of the ACL has been found to mainly occur in the subsynovial layer and close to the insertion sites.^{5,26} Certain studies have found that very few mechanoreceptors are present within the ACL. These receptors account for the role of the ACL in knee proprioception and are known as Ruffini receptors. Ruffini receptors are slow-adapting and function as stretch receptors and nociceptors. These nociceptors may play a crucial role in the healing of grafts and tissue homeostasis. The rapidly-adapting receptors are known as Pacinian corpuscles which sense motion in any position.^{5,16} In short, the mechanoreceptors of the ACL identify changes in motion, position, acceleration and the angle of rotation of the knee joint.⁷

The main function of the ACL is to connect the femur and the tibia to one another.⁵ The ACL limits the posterior rolling of the femoral condyles on the tibial plateau during flexion of the knee joint. It also prevents the femur from displacing posterior on the tibia and hyperextension of the knee joint.^{4,21,22} The ACL has been revealed to be primarily restricted to anterior tibial translation and restricted secondarily to internal rotation of the non- and weight-bearing knee.^{5-7,16,20,27,28} The ACL has shown to produce internal tibial rotation when a load is applied across the tibiofemoral joint. An increase of the force in the ACL is observed when internal tibial torque is combined with anterior tibial force in almost full extension of the knee. During forced hyperflexion, the force is larger in the ACL than in the PCL. MRI and three-dimensional computer modelling techniques have recently been used in *in vivo* studies to examine the morphological changes which ligaments undergo during elongation and rotation. The length of the ACL decreases 10% at 90° flexion when compared to complete extension. The ACL shows 20° internal rotation at 30° flexion of the knee. At lower flexion angles the ACL appears to have a more important weight-bearing role.¹⁰

Examination of the embryological development of the ACL shows that during the eighth week after gestation a distinct number of ACL fibres are visible. The fibroblasts are already aligned to the axis of strain of the ACL. The cruciate ligaments comprise of numerous immature fibroblasts by nine weeks. Scanty cytoplasm and fusiform nuclei can be observed. An increase in vascularity can be expected over the next few weeks. The ACL of fetuses with a gestational age of 24 - 40 weeks showed an almost identical appearance to the adult knee.^{5,9} The foetal ACL tissue is more cellular and vascular than the ACL of an adult. The ACL consists of two distinctive bundles, the anteromedial and posterolateral bundles (Fig. 2.5).^{5,7,9,10,20,22,29} These two bundles are already detectable as early as the 17th gestational week.^{5,7} In the foetus it has been found that these bundles are separated from one another by a connective tissue septum.¹⁰

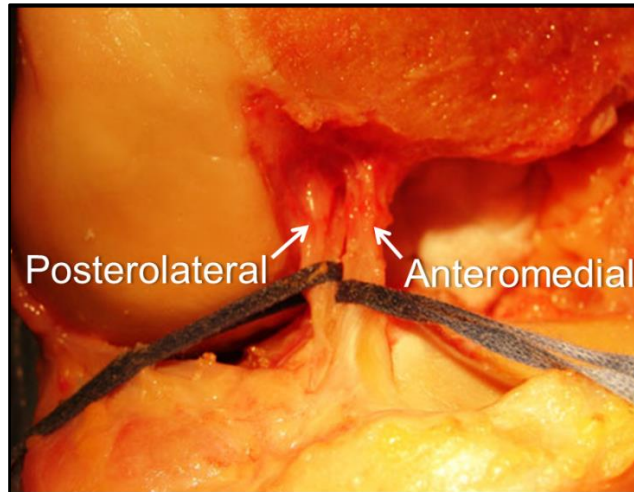


Fig. 2.5: The anteromedial and posterolateral bundles of the ACL.⁷

However, some studies have proposed the existence of a third intermediate bundle.^{5,7,9,22,30,31} Although disagreement exists on the number of bundles present, there is general consensus that the ACL comprises of distinct functional bands.¹⁰ Dividing the ACL into two bundles makes it easier to understand the function and contraction of the ligament. These bundles play important roles in controlling the stability of the knee. The anteromedial bundle controls anterior tibial transformation. Its origin is in the most proximal region of the femoral origin and it inserts on the most anterior aspect of the anterior intercondylar area.^{5,7,9,20,22,31} The posterolateral bundle is largely in charge of stabilising the knee during rotation. It restricts the internal rotation of the lateral tibial plateau.³² The posterolateral bundle originates from the distal aspect of the femoral origin and inserts posterior to the anteromedial bundle (Fig. 2.6).^{5,7,20,22,29}

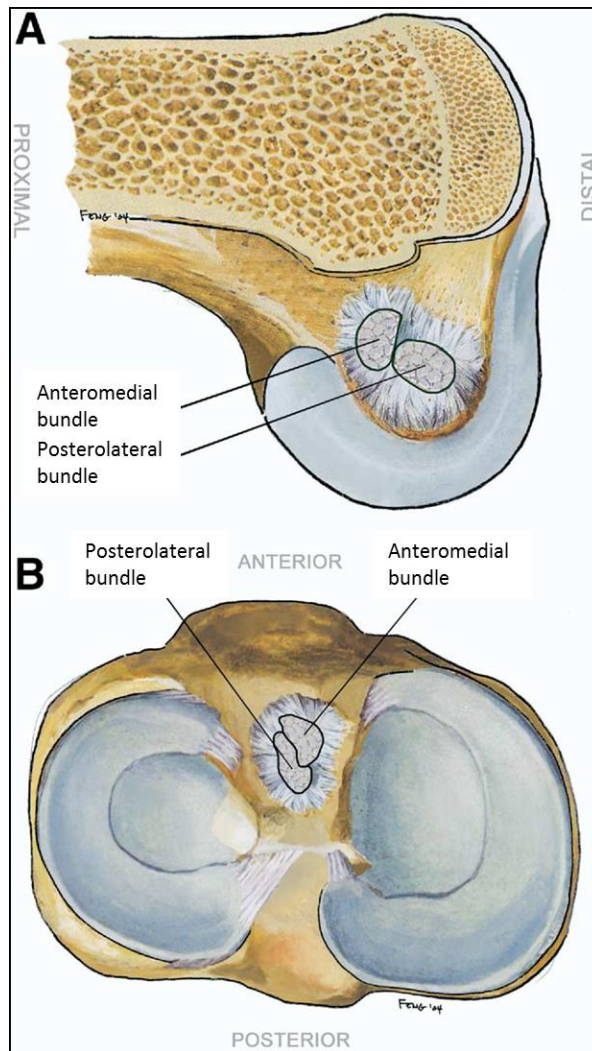


Fig. 2.6: The origin (A) and insertion (B) sites of the anteromedial and posterolateral bundles of the ACL.¹⁶

The length and position of these bundles differ depending on the angles of knee extension and flexion.¹⁰ During knee extension the posterolateral bundle is tense and the anteromedial bundle fairly relaxed. As flexion occurs, the femoral attachment will become more horizontally oriented. This will cause the anteromedial bundle to tense and in return the posterolateral bundle will relax.^{5,7,9,16,30} The ACL lengthens up to 3mm when the knee is extended. Flexion from 0 - 30° shortens the anteromedial bundle, but continued flexion from 30 - 70° lengthens it back to its original length. Flexion beyond 70° continues to lengthen the anteromedial bundle past its original length until maximum strain is achieved. On the other hand, the posterolateral bundle achieves maximum length and strain at full extension of the knee.¹⁰

From extension to flexion the posterolateral bundle shortens.¹⁰ The anteromedial bundle elongates with about 3.3mm at 90° of flexion while the posterolateral bundle reduces its length by 1.5mm.^{5,22} Tibial rotation showed no significant effect on ACL fibre length. However, internal tibial rotation increases the fibre length more than external rotation, most obviously at approximately 30° of flexion.^{5,20,30} Internal rotation at 90° has been shown to lengthen the posterolateral bundle by a mean value of 2.7mm.^{5,22,33} The anteromedial bundle extends less than the posterolateral bundle during flexion. The posterolateral bundle carries the greatest load from 0° - 45° of flexion with a maximum at ±15°. It bears about 35% at 90° flexion. The anteromedial bundle on the other hand, carries its greatest load between 60° and 90° flexion of the knee. It bears 45% of the load of the ACL during flexion and 30% during extension.³²

2.4 Histology of the anterior cruciate ligament

Previous studies have examined the normal macro-anatomy of the ACL, but there seems to be less information on the histological composition of this ligament. The graft used to replace a torn or ruptured ACL needs to take over the functions of the intra-articular ACL.

The ACL is a band of dense connective tissue which, at microscopic level, cannot be separated into two bundles.^{5,7,20,34} The ACL consists of thick, closely packed, longitudinally oriented collagen bundles. A small number of fibroblasts are scattered in between the collagen fibres and their cell bodies appear elongated (Fig. 2.7).^{5,25,35} The collagen fibres show a sporadic crimp arrangement or rather take a helical path around the axis of the ligament, which is surrounded by a thin synovial membrane sheath.^{30,36,37} The collagen fibres of the ACL are surrounded by soft connective tissue which also divides the fibres into numerous fascicles (Fig. 2.8).



Fig. 2.7: Elongated spindle-shaped fibroblast found in the central part of the ACL.²⁰

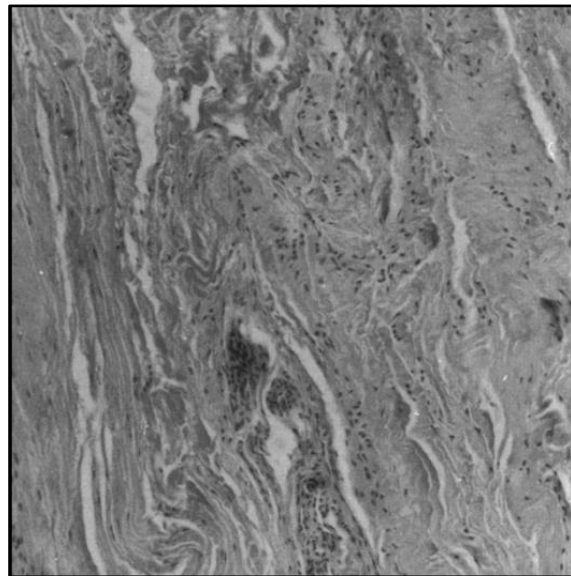


Fig. 2.8: ACL fibre bundle subdivided into multiple fascicles.³⁵

The main collagen found within the ACL is Type I collagen. Type III collagen is found within the loose connective tissue.^{5,7,9,22,25,35} The ACL shows an irregular fascicular arrangement which gives it a multi-axial organisation.³⁵ This arrangement enables the ACL to withstand stresses in multi-axial planes with various tensile strains. Parallel arranged fibres and spindle-shaped cells are present in the ACL with perpendicular fibres in some areas. An undulated crimp and helical wave pattern is found. The crimp pattern fibres withstand small loads of tensile stretch while the helical fibres resist multi-axial or unpredictable loads.³⁷ The anterior part of the distal third of the ACL contains tenocytes and chondrocyte-like cells (Fig. 2.9). The presence of chondrocytes can be explained as a functional adaptation to the stresses occurring between the ligament and bone.^{5,25}

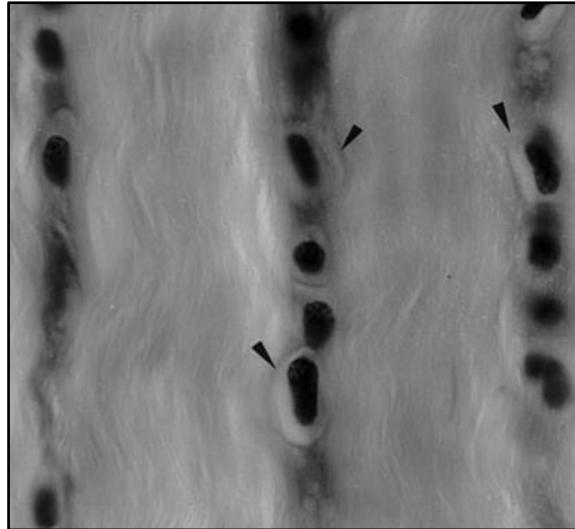


Fig. 2.9: Chondrocytes in between the longitudinal collagen fibres. The chondrocytes have a round cell shape and they lie in rows.²⁵

The femoral origin and tibial insertion sites of the ACL comprise of four layers (Fig. 2.10). Layer one consist of ligament fibres. The second layer contains fibrocartilaginous cells (non-mineralised/uncalcified cartilage zone). Layer three is known as the mineralised (calcified) cartilage zone and the last layer contains the mineralised fibrocartilage that inserts into the subchondral bone plate.^{5,7,10,17,18,22,38}

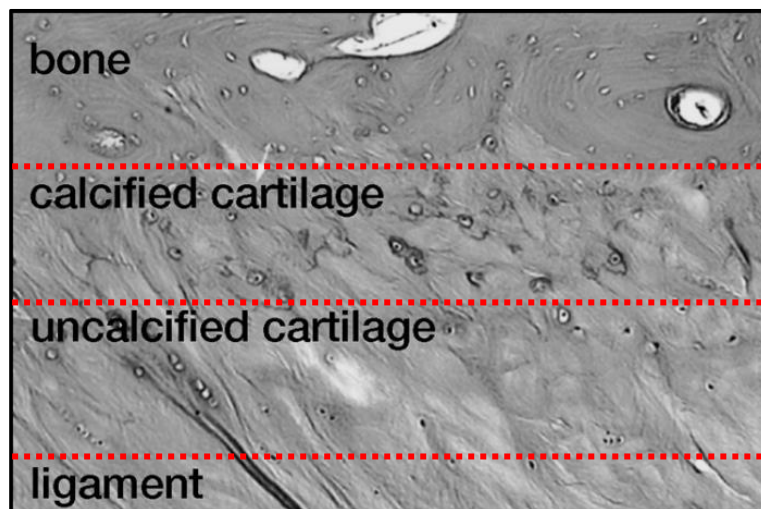


Fig. 2.10: A histological section of a direct insertion showing the four layers of insertion: ligament proper, uncalcified fibrocartilage layer, calcified fibrocartilage layer, and bone.³⁸

This specific composition of the insertion sites of the ACL allows the graduate change of stiffness from the bone to the ligamentous tissue and can inhibit the stress concentration at the attachment site.^{5,22,25,38} The ACL consists of three histological zones which are composed of spheroid, ovoid and fusiform cells (Fig. 2.11).^{20,39} The proximal part of the ACL is highly cellular and rich in spheroid and ovoid fibroblasts with some fusiform fibroblasts.^{20,40} The spheroid and ovoid cells seem to have chondroblastic characteristics.^{17,18,39} The central part contains fusiform fibroblasts and dense collagen fibres. The central part of the ACL is also called the fusiform zone because of the longitudinally oriented cells with fusiform-shaped nuclei (Fig. 2.12). Longitudinal blood vessels are also found within this zone. The central part of the ACL displays a high collagen density, low cellularity and elongated fibroblasts. The distal part is rich in spheroid to ovoid fibroblasts and chondroblasts with a low collagen bundle density.^{20,40}

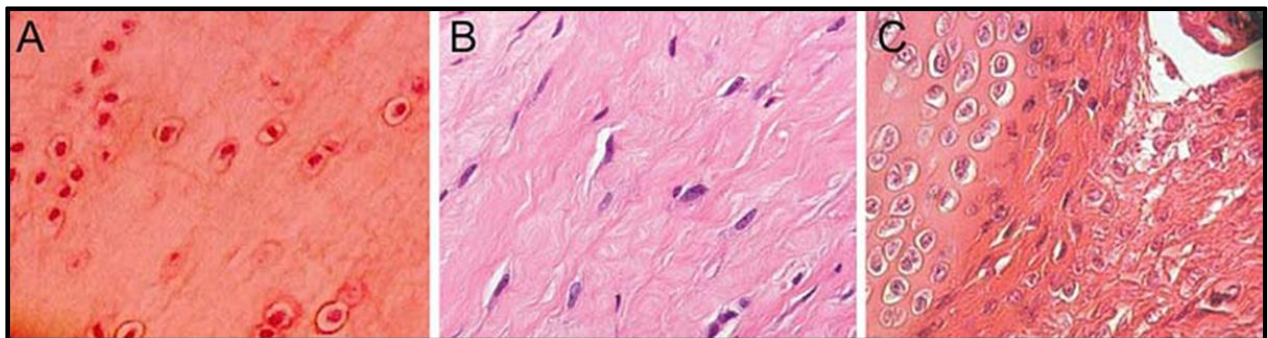


Fig. 2.11: The three histological zones of the ACL. The proximal part which is highly cellular with fibroblasts with spheroid and ovoid nuclei (A), the central part with fusiform nuclei (B), and the distal part with spheroid and ovoid nuclei (C).²⁰

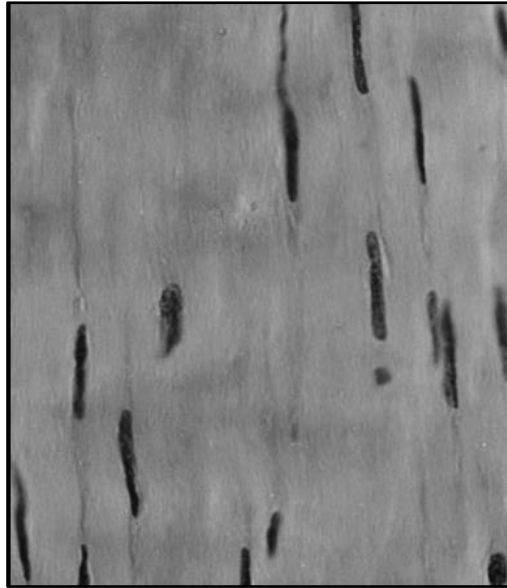


Fig. 2.12: Fibroblasts with fusiform nuclei.²⁵

Type I collagen fibres are oriented parallel to the longitudinal axis of the ACL and are in charge of the tensile strength of the ligament. Within the fibrocartilaginous regions, Type II collagen is found. This collagen type is found in areas where the ligament is exposed to tendon pressure. Type III collagen is located in the loose connective tissue of the ACL and divides the Type I collagen bundles. Type III collagen is important for the flexibility of the ACL. Type IV collagen is present in the vascular basement membranes of the ligament and, lastly, Type VI collagen serves as a gliding component between the fibrillar units of the ACL.²⁰ A decrease in cell density and vascularity is found from proximal to distal while the fibroblast nuclei become increasingly more spherical.⁴¹ The arrangement of collagen fibres also differ at the proximal, distal and central regions.³⁷

Murray *et al.*⁴¹ determined that age and gender do not influence the number of cells or the blood vessel density of the ACL. The ACL is incapable of regenerating itself after injury due to its poor blood supply.²⁴ Scapinelli²⁴ determined the intrinsic vasculature of the ACL. They found that the endoligamentous vessels are lodged in the interfascicular septa. These vessels are accompanied by venules and thin nerve fibres. The intrinsic vessels in the proximal and central parts of the ACL indicate a descending course while those of the distal aspect, ascend. This explains the avascular area found between these two territories.²⁴

2.5 Anterior cruciate ligament injury

Almost half of all ligamentous knee injuries are ACL injuries.¹⁰ In fact, the ACL is possibly the most frequently and totally disrupted knee ligament.²⁷ Most ACL injuries are associated with sports activities with football and skiing at the top of the risk list. An ACL injury could be career-limiting to almost all professional sports men and women. Successful ACL reconstruction can change the lives of many injured sports patients for they would not have been able to return to their sports otherwise.^{3,10}

Female athletes have a much greater occurrence of ACL injury when compared to their male counterparts.^{3,9,10,42,43} Delee *et al.*¹⁰ have documented the occurrence of ACL rupture in females to be three to six times higher than in males, while Jamison *et al.*⁴³ have found it to be 11 times higher. Shelbourne *et al.*⁴⁴ found that female football players have an ACL injury rate of 2.4 times that of male football players and 4.1 times in basketball players. Anderson *et al.*⁴² reported women to suffer two to eight times the number of non-contact ACL injuries incurred by men.^{3,42} This may be due to anatomical, biochemical, neuromuscular and hormonal differences. Anatomically, the width of the intercondylar notch, knee alignment when standing, body mass index and joint and ligament laxity may also play a role.^{10,42} The width of the intercondylar notch is smaller in females than in males.^{5,9,42,44} Shelbourne *et al.*⁴⁴ described a narrower intercondylar notch as a risk because impingement of the ACL may occur during hyperextension of the knee. However, Anderson *et al.*⁴² concluded that ACL size does not vary in direct proportion to the intercondylar notch size and it is therefore not the reason for the higher occurrence of ACL injury found in female athletes. Another factor may be the considerably reduced cross-sectional area of female ACLs. They are narrower, shorter and have a smaller volume than in males.⁴³ However, with corrections for body weight, no significant difference was found for the area of the ACL between males and females.⁴² This could be explained by the fact that everything is proportionately smaller in females than in males. Biochemical and neuromuscular differences cause ACL injuries when the knee is loaded with a high dynamic load. The stability of the knee is also influenced by these differences. Females tend to show increased activation of the quadriceps when compared to the

hamstring. This functional imbalance causes the ACL to gain extra load.¹⁰ Female ACLs also show lower mechanical properties, for example modulus of elasticity.⁴³ Undoubtedly, extrinsic differences between males and females contribute to the sex difference in ACL tear rates, but injury is also caused by several interrelated intrinsic factors such as body fat, quadriceps muscle strength and the size of the ACL. Although various factors may influence the likeliness of females to sustain ACL tears, no conclusive explanation has been identified.⁴²

The mechanism of injury of the ACL usually takes place when sudden deceleration or pivoting occurs or even the landing of an athlete after jumping. The ACL can tear when force is exerted anteriorly against the femur with the knee in a semi-flexed position, when hyperextension has occurred, or when the femur and tibia twist in opposite directions.^{1,3,4} In France, five of the sports responsible for 90% of ACL injuries are football, skiing, rugby, basketball and judo. Severe external rotation, internal rotation and hyperextension of the knee can injure the ACL.⁴⁵

Injury to an extra-articular ligament will lead to haematoma formation and an inflammatory response. Granulation tissue will form which will lead to the formation of fibrous tissue to restore the function of the ligament. This is not the case with the intra-articular ligaments of the knee joint. ACL injuries are difficult or even impossible to heal spontaneously. There are many possible reasons for this. The ACL is an intra-articular ligament and is enclosed by a thin synovial sheath. Injury to the ACL will influence this sheath as well. Haemorrhage caused by the injury will spread throughout the joint cavity and prevent fibrous tissue formation. Therefore the ligament cannot be restored. With partial injuries there is a possibility that the synovial sheath can remain intact. This will allow blood clot formation and initiate fibrous tissue formation for restoration. The cytokine profile of this intra-articular area may also add to the poor healing properties of the ACL and can lead to the development of osteoarthritis. The cell migration of the cruciate ligament cells is slow when compared to that of the collateral ligaments.^{10,38}

Because of the poor healing ability of the ACL, reconstructive surgery is often the only way to regain knee stability. A ligament graft is transplanted to replace the torn ligament and is initially subjected to avascular necrosis and inflammation. Revascularisation of the remaining graft tissue occurs after the fibroblasts have died off. Migration and repopulation then occur where after graft remodelling and the remodification of collagen follows. The collagen aligns similar to the original ACL. It is important to realise that the transplanted graft will never fully resemble the normal ligament, although previous studies have found it to resemble the native ACL histologically after 12 months.^{10,21}

2.6 Evaluation of anterior cruciate ligament injury

A patient needs to be properly evaluated to diagnose and treat the ACL injury. Some of the more common causes of ACL injuries include direct contact and indirect non-contact injuries such as rotational movements or rapid deceleration. Indirect non-contact injuries are more common.¹⁰

The patient can usually recall a popping or tearing sensation at the moment of the injury. Patients with ACL injury cannot bear weight on the injured leg. Ligament injuries are also identified by post-traumatic swelling which is indicative of the disrupted blood supply of the ligament. Swelling is observed within 12 hours after the injury occurred.^{10,45}

A physical examination is required to establish a positive diagnosis of ACL injury. The sooner the examination is done, the more accurate the diagnosis. Step one of the examination processes is to observe the knee and determine if swelling or misalignment is present. Misalignment can indicate a fracture or knee dislocation. Step two is to palpate both knee joints in order to compare the normal and injured knee. Palpation is required to detect an effusion missed with inspection and the degree thereof. The surrounding knee structures are also examined for injuries. Step three is to test knee function by performing active and passive range of motion tests. This will determine whether loss of motion has occurred which may be caused by

pain, large effusion, mechanical block or ineffectual extensor mechanism. The presence of haemarthrosis will indicate ligament injury. Fat globules in the synovial fluid of the knee joint will indicate a knee fracture. If a mechanical block is present, radiographs or MRI scans may be required to enable a proper diagnosis. Step four is to test the stability of the knee. It includes testing anterior, posterior, valgus, varus and rotational stability.¹⁰ The Lachman test or anterior drawer test is used to test for ACL injury (Fig. 2.13). The knee is flexed between 60° and 90° with the foot held still and the patient in a supine position. Anterior stability is tested by applying an anterior force manually to the tibia while the distal femur is kept still with the opposite hand. The degree of anterior translation of the tibia is then assessed in relation to the femur. A comparison is made between the normal and injured knee and the degree of translation is then categorised according to the grades of laxity.^{10,45}



Fig. 2.13: Anterior drawer test with the knee at 90° flexion.⁴⁶

In 10 - 28% of all ACL injuries, partial tears are present. It is much more challenging to diagnose partial tears and therefore a combination of tests are used. If the posterolateral bundle is involved in the partial ACL tear, pivot shift tests are administered to test the integrity of this bundle. A partial ACL tear involving the anteromedial bundle will be tested with the anterior drawer test and Lachman test to examine sagittal plane stability.¹⁰ MRI scans are helpful in the correct diagnosis of a partial tear of the ACL. Suggestive signs are the absence of bone bruising, increased signal intensity of the ACL and the inability to identify all fibres.^{10,21} It is very difficult to treat partial tears, because age, physical activity level, other injuries, laxity degree and symptomatic instability all play a role. Partial ACL tears can be treated non-operatively in patients who are willing to follow a program of hamstring strengthening, activity modification and brace wearing. Reconstructive surgery is the

other option. It is possible to perform single-bundle reconstruction, where only the torn bundle is replaced and the undamaged bundle remains functionally intact.¹⁰

2.7 Radiographic evaluation

There is very high demand for people participating in sports for quick and accurate diagnosis. This is because even the smallest interruption in their training programme can result in performance setback. These athletes often receive the most sophisticated diagnostic programmes and it is therefore crucial to identify the limitations and possibilities of the imaging modalities and evaluation processes used.⁴⁷ Rapid and accurate evaluation and pre-operative planning seem essential in these cases. MRI scans can assist in the estimation of the graft sizes required for reconstruction, which may be helpful to predict graft insufficiencies and take the necessary precautions. It will also be beneficial if radiographs can be used to assist in pre-operative planning, especially if it can spare the patient the cost and time of taking MRI scans as well.

MRI has become the imaging modality of choice for assessing acute knee injuries due to its soft tissue contrast, multi-planar capabilities and the lack of ionising radiation.^{21,48} It has largely replaced diagnostic arthroscopy. MRI scans are helpful in assessing the ACL for fluid and partial tear presence. Previous studies have confirmed the reliability of using MRI scans to assess ACL integrity. The large number of patients evaluated requires the use of a rapid and accurate technique for ACL assessment. The ACL is best visualised on sagittal images (Fig. 2.14), but because of its oblique course, two or three sagittal sections should be imaged. Oblique sagittal images have been found to be more exact than straight sagittal images.⁴⁸

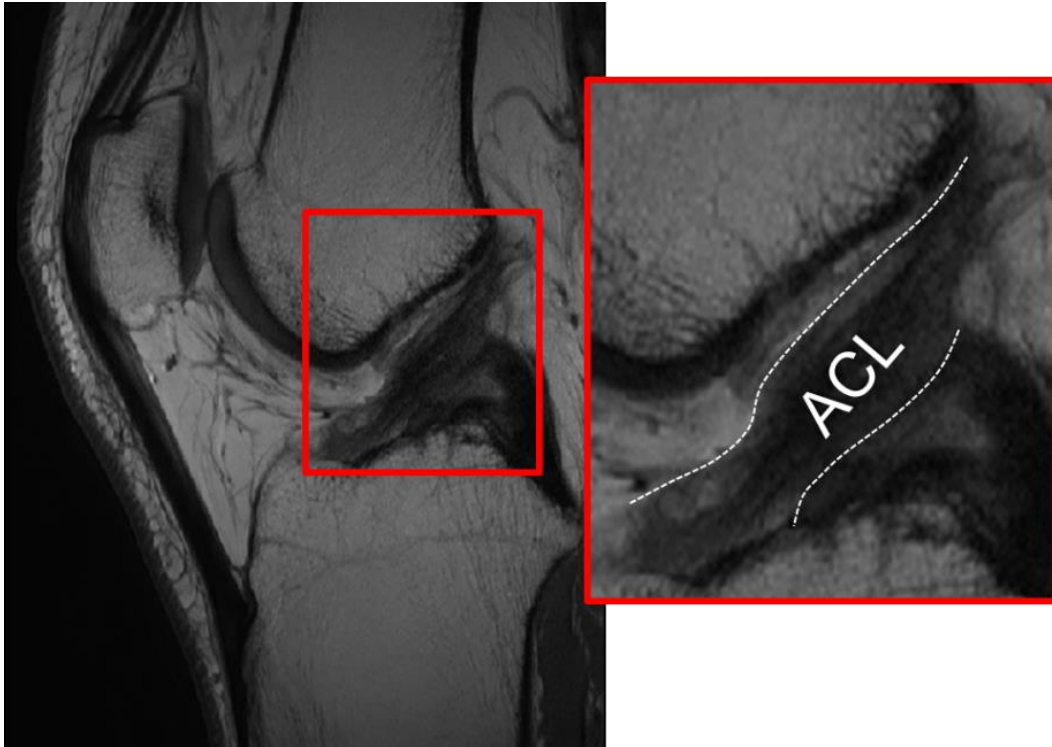


Fig. 2.14: Sagittal MRI scan of the ACL to visualise its entire length.

An ACL tear is identified on MRI when a rupture in the fibres, or a soft tissue mass in the notch is visible with high-signal characteristics from oedema and haemorrhaging. Partial ACL tears are identified by increased signal, thickening or redundancy in the ACL. Suspected partial and complete ACL tears are usually evaluated arthroscopically.⁸ Radiographs, computed tomography scans and MRI scans are also useful in determining the tunnel positions for ACL reconstructive surgery.⁵

Scanlan *et al.*⁴⁹ used MRI to determine whether the contralateral knee could be used to predict the placement of the ACL graft in the injured knee. They noted that the anatomy of the ACL in the reconstructed knee, compared to that in the contralateral knee, was not significantly different when compared to the left and right knees of the healthy control group. Small side-to-side differences exist in the footprint of the ACL between healthy and injured patients. Reconstruction therefore should be performed and evaluated on an individual basis. Differences in imaging modalities may explain the variations found in studies regarding side-to-side differences between the injured and contralateral knee. This study provides evidence of small side-to-side differences.

Intra- and extra-articular lesions, for which clinical examination and arthroscopy cannot account, should be investigated using MRI. A study by Odgaard *et al.*⁴⁷ investigated the clinical significance of MRI results on treatment and repeated clinical examinations. They concluded that clinical examination alone may leave many lesions insufficiently treated, whereas findings based on MRI evaluation alone, can lead to the over-estimation of the number of lesions that require treatment. It is therefore important to correlate the findings from both the clinical examinations and MRI. The expertise of the surgeon plays an important role in the evaluation and treatment of the injury. Contrary to this, Kockabey *et al.*⁵⁰ found no statistically significant difference between MRI and clinical examinations. They concluded that a well-trained qualified surgeon can safely rely on clinical examination for the diagnoses of ACL and associated meniscal injuries.

A study by Ichiba *et al.*⁵¹ found that ACL size can be predicted by measuring the anteroposterior diameter of the tibial attachment site of the ACL (fovea) on a sagittal MRI scan. They confirmed that ACL size could be predicted by using physical characteristics of the patients such as weight and height, although they were unsure of which characteristic was the best predictor. The results of this study may prove useful in the pre-operative planning for ACL reconstruction. A limitation of this study was that the ACL fovea is not always clearly visible on MRI scans. Brown *et al.*¹¹ conducted a MRI study on live American patients in order to use patient height to determine appropriate graft length for ACL reconstruction. Brown *et al.*¹¹ found a strong correlation between ACL length and patient height. They concluded that patient height can predict the required length of the PL graft used for ACL reconstruction. This would assist in more time efficient pre-operative planning.

Imaging modalities are currently used for ACL reconstruction preparation to decide on techniques and the graft size required. MRI scans are routinely taken before any ACL reconstruction and used to inspect the knee before surgery. Presently, the graft length is not determined pre-operatively, but only during surgery. The ability to determine the length of the graft ligament required for replacement of the ACL in advance will facilitate easier and more time efficient reconstruction and reduce complications after surgery. However, MRI machines are often scarce resources and it would therefore be helpful to have another resource available in the assistance of

ACL assessment before ACL reconstructive surgery. Radiographs are readily available and are also routinely taken for knee injuries. It would therefore be very helpful if radiographs could be utilised for pre-operative planning without the need to rely on MRI scans.

2.8 Treatment options

People over 40 years of age, have become more active and this has led to an increased occurrence of ACL injuries. In the past, operative treatment was not considered, but it has been shown that older patients too, benefit from ACL reconstruction. It is important to address the ACL deficiency soon after the injury, before chronic degenerative changes set in. Patients over 40 years old, who wish to remain active, are recommended to undergo ACL reconstruction. However, patients who are prepared to accommodate a less active lifestyle, can manage with an ACL-deficient knee, without surgical intervention.¹⁰

Symptomatic instability of the knee, together with a proven ACL tear, are indications for ACL reconstruction.^{3,15,52} Treatment options include non-operative interventions, but the preferred method of ensuring future knee stability is ACL reconstruction.^{2,7,10,15} It is often found that other knee injuries accompany ACL tears^{6,10,22} such as meniscal and medial collateral ligament (MCL) tears. It is essential to preserve the meniscus, for it plays a role in the stability of the knee, load transmission and preventing long-term arthrosis.¹⁰ Meniscal repairs done together with the reconstruction of the ACL have a higher healing rate.^{10,52} The formation of fibrin clots and haemarthrosis are the reason for this. MCL injuries are mostly treated with non-operative bracing.¹⁰ It is also wise to inspect the PCL and popliteal tendon for the presence of PCL or posterolateral corner injuries.⁵²

Chronic ACL deficiency can be the precursor for chronic instability of the knee and this increases the risk of chondral or meniscal injuries. This can cause further intra-articular damage and even lead to osteoarthritis.^{2,3,10,21,22,27,45,48} The most common complaint of patients with ACL deficiency is that the knee repeatedly gives way. This can cause the patient to be less active.¹⁰

2.9 Operative treatment

In cases where reconstructive surgery is required to correct and re-establish the stability of the knee joint, surgery can only be carried out three weeks after the injury. It has been shown that joint stiffness can be the consequence of earlier surgery due to scarring within the joint. Surgery should only commence after the swelling has passed and the knee can flex and extend.¹⁰

Early primary surgical repair was thought to be capable of re-establishing knee stability, but 5 - 15 year follow-up showed poor results with physical and radiographic examinations. Augmentation procedures were then applied where hamstring or iliotibial tendons were used for reconstruction. Knee stability has shown to have improved, as did knee function, but long-term follow-up still indicated deterioration.¹⁰ A high incidence of joint degeneration has been reported with the long-term follow-up of patients after ACL reconstruction. Although the mechanisms that contribute to joint degeneration are probably multifactorial, some studies propose that the inability of reconstruction to repair normal joint kinematics, may lead to the development of osteoarthritis.⁵³ Prosthetic ligament reconstruction, which prevented the formation of biological reconstructive tissue, became popular. Decreased morbidity, stronger grafts and increased rehabilitation were found with ligament augmentation devices. However, complications such as continual effusions, reactive synovitis, infection and delayed maturation occurred.¹⁰ Previous studies found that the most common cause of ACL reconstructive failure is the misplacement of the grafts. This directed studies toward fibre length changes and the concept of isometry. Isometry implies that the distance between the attachment points of the graft remains constant during movement of the knee. Theoretically this might work, but in reality, any form of loading of the knee will disrupt isometry. This was confirmed by Amis and Dawkins.³⁰ Therefore, it is important to attempt to replicate the macroscopic arrangement of the ACL fibres. A good start would be to determine the length of the ACL before injury.⁶ ACL reconstruction procedures have been improved over time: from open surgery procedures, to the arthroscopic two-incision technique, to the one-incision technique. Double-bundle ACL reconstruction developed after the conventional single-bundle

reconstructive surgery. Studies have led to better understanding of the time of surgery, graft selection, harvesting techniques, tensioning, ACL tunnel placement and fixation.¹⁰

Intra-articular reconstruction to restore ACL function is performed by using autograft (a patient's own tissue) or allograft (a graft of tissue obtained from a donor of the same species) material.¹⁰ Many factors influence the optimal graft selected for ACL reconstruction. These factors include patient age, activity level and concerns with donor site morbidity.¹² The graft selected for reconstruction depends on the surgeon, but it should comprise of similar structural properties than the original ACL.¹⁰ The tendon graft needs to match the properties of the native ACL to ensure the best surgical outcome after ACL reconstruction. These properties can be differentiated as either structural or mechanical properties.²² It is important that the chosen graft allows safe fixation, sufficient graft tensioning, excellent biological incorporation and minimum donor site morbidity. The options for ACL autografts include: BTB, quadriceps tendon, quadrupled semitendinosus and gracilis hamstring (HS) tendon grafts. Allografts used are BTB, HS, quadriceps-, achilles- and tibialis anterior and ~posterior tendons.^{10,12,16,22,38} Most ACL reconstructions are performed with BTB or HS autografts. However, reconstructions using allografts resulted in good surgical outcomes and less donor site morbidity.¹⁶

2.10 Patellar ligament and graft

The PL is used for ACL reconstruction. The PL originates as an extension of the quadriceps femoris tendon at the lower pole of the patella and then inserts onto the tibial tuberosity.^{10,54} The PL contains fibroblasts arranged between the parallel collagen bundles.

A common source of grafts used for ACL reconstruction is the BTB graft, as first described by Palmer⁵⁵ in 1938. This graft is harvested from the middle third of the PL in the long axis (Fig. 2.15).^{7,11-15} This technique was initially described by Jones^{56,57} in the 1960s. The BTB graft is considered to be the graft of choice. This is due to its

good track record, graft strength, bone-to-bone healing, early osseous integration and firm fixation.^{10,16} It has also been reported that a 10mm wide BTB graft has comparable structural properties to the native ACL. This graft is assumed to be strong enough to be used as an ACL replacement graft.^{16,22}

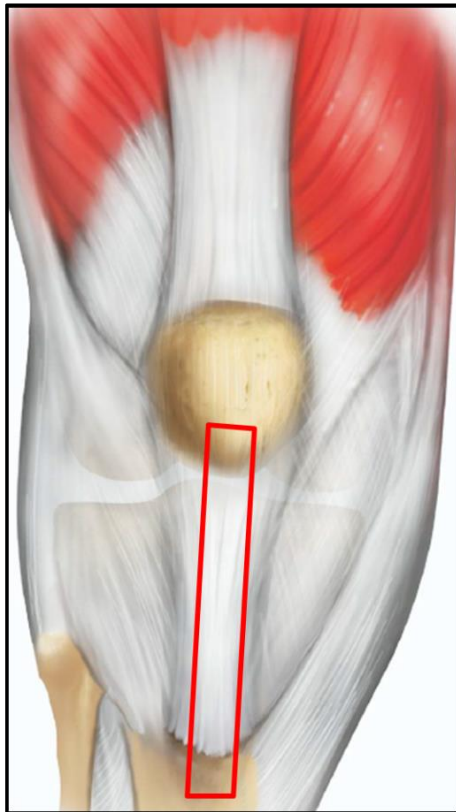


Fig. 2.15: The red outlined rectangle indicates the middle third of the PL harvested for a BTB graft.⁵⁴

A BTB graft is harvested using an anterior approach. The end of the table is dropped in order to let the knee go down into flexion, or a leg holder is used.¹³ The incision is made in the midline from the inferior pole of the patella to just superior to the tibial tuberosity.⁵² The paratenon is exposed and then dissected from the underlying tendon fibres in order to reveal the PL.¹³ The central 10mm (middle third) of the ligament is removed.^{13,54} The dissection should be continued proximally across the patella and distal to the tibial tuberosity for a further 30mm.⁵² The reason for doing so is to mark the sites of bone cuts for the harvesting of proximal and distal blocks. Two 10mm deep holes are drilled with a 2mm drill in each area of bone between the dissected borders. These holes are used to pass sutures for controlling the graft at

the insertion site. A 25mm block of bone is dissected from the patella using a narrow oscillating saw and osteotome. A trapezoidal graft shape is then created by placing the osteotomes at 45° towards the midline while cutting. If the graft is too deep it can lead to a patella fracture, and too shallow will prevent fixation of the graft. The ideal is a block of 25mm long, 8mm wide and 5 - 8mm deep. The block is trimmed to a uniform size and two heavy sutures are passed through the drilled holes. This procedure is repeated to harvest the bone block from the tibial tuberosity.^{2,52,54} An autograft is preferably used in order to prevent graft rejection.

ACL reconstruction using a BTB autograft does not re-establish the kinematics of the knee under physiological loading circumstances.¹⁰ Using a BTB graft has proved to successfully limit anterior tibial translation under anterior tibial loads. However, rotational load showed a lesser degree of success. Similar results were found with HS grafts.²² Both ACL bundles need to be included in the ACL reconstructive graft in order to re-establish the translational and rotational kinematics of the knee.¹⁰ It is commonly believed that BTB grafts integrate faster and more predictably than soft tissue grafts.^{16,38} BTB grafts have proved to be 168% stronger than the original ACL.¹⁰ These grafts can also withstand higher loads and display increased stiffness when compared to HS grafts.^{16,38} Butler²⁷ concluded that the BTB graft is the only graft which exceeds the strength and stiffness of the ACL. All other grafts have shown to be weaker than the original ACL.¹⁰

Patellar BTB graft size mismatch is one possible pitfall with the performance of ACL reconstruction. This problem occurs more often in allograft than autograft reconstruction. The problem is that the tendon distance between the tibia and femur is either too short, or more often, too long. Using a graft of the incorrect length can jeopardise regaining knee stability.¹⁰ A long BTB graft can lead to an excessively steep tibial tunnel which may prevent the correct placement of the posterior femoral tunnel.¹⁵ The correct measurement of the graft is therefore essential to allow proper execution of the surgical procedure. It will prevent the erosion of the ligament caused by impingement by the screw used in ACL reconstructive surgery. Pre-operative determination of ideal graft length to replace the ACL will be of great value to the surgeon to achieve optimal ACL reconstruction using the PL (BTB graft).¹⁰

2.11 Graft healing and post-operative complications

The healing of the graft involves the attachment site of, and the ligament itself, as well as the revascularisation and integration of the graft. BTB grafts take about six weeks to heal while soft tissue grafts take longer (8 - 12 weeks), as they do not have bone blocks. Graft healing progresses through phases. The first phase is the inflammatory phase, where the graft undergoes inflammation and necrosis. The donor fibroblasts undergo cell death while the host fibroblasts will migrate and repopulate. Phase two involves the revascularisation of the graft and the strength and stiffness of the graft will decrease within 20 days after surgery. During the last phase, the graft almost reaches its original strength, as collagen maturation occurs. It has been found that allografts incorporate at a slower rate than autografts and this can increase the chance of ruptures occurring.^{10,38}

Donor site complications are rare, and mostly occur in autograft BTB grafts. Patella fractures, PL ruptures, patellar tendonitis, quadriceps weakness and local numbness can be experienced.^{10,12,16} Rupturing of the PL is a very rare complication, but special care should be taken to determine the middle third of the ligament when harvesting the graft. The PL may shorten due to suturing of the ligament after harvesting the graft and it is preferred to also close the paratenon. This will assist in healing and minimising scarring of the overlying skin. Up to 50% of BTB harvests have resulted in anterior knee pain, but this is not proven to be due to graft harvesting. HS autograft harvesting may cause injury to a superficial branch of the saphenous nerve.¹⁰ Allograft use has increased over the last 20 years. This is due to decreased morbidity, decreased operating time, the availability of grafts of specific sizes, preservation of the flexor and extensor mechanisms of the patient, enhanced aesthetics and a dependable source of graft material. Unfortunately, allografts show tunnel enlargement, incorporates slowly and the risk of disease transmission is always present.^{10,16}

Graft tensioning throughout ACL reconstruction is a difficult task. Adequate tension is required to restore the anteroposterior stability of the knee, but too much tension can lead to the failure of graft fixation and graft stretching.^{10,38} Graft tension is influenced by the angle of knee flexion, the rotational position of the knee and the type of graft used. Previous studies have concluded that no significant difference was found between two tension levels of BTB grafts. HS grafts only showed a small degree of increased knee laxity. It is important to preload the graft in order to prevent the loss of graft tension.¹⁰

It was found that ACL reconstruction using BTB grafts showed a statistically significant decrease in the rate of knee failure and laxity, and supplied a stable knee to the patient. HS grafts on the other hand, have shown to decrease the occurrence of anterior knee pain and the rate at which arthrofibrosis occurs. Range of motion, complication and failure rates and Lachman tests showed two major differences between these two graft types. Pain might still be experienced when kneeling with the use of a BTB graft and HS grafts have shown slightly increased knee laxity. Correct tunnel placement is very important. Should the graft not be placed at the optimal position, complications are more likely to develop. Misplaced tunnels are one of the most common causes for the revision of ACL reconstructions.¹⁰

The relationship between incorrectly placed grafts and graft failure, as well as the need to replicate normal knee function after ACL reconstruction, have redirected the goal of reconstruction outcomes toward re-establishing native ACL anatomy.⁵³ The ideal graft should replicate the anatomy of the native ACL. It should comprise of similar biomechanical properties to allow secure fixation of the graft, rapid biological incorporation, enhanced rehabilitation and minimum donor site morbidity.¹⁶ New techniques are constantly being developed. Replacing both ACL bundles has great potential to re-establish the normal stability of the knee. Determining the length of the ACL before surgery will assist in the process of restoring the native anatomy of this ligament.

2.12 Pre-operative planning

Successful graft reconstruction requires knowledge of the morphological characteristics (macro- and micro-anatomy) of the PL and the ACL, as well as of the relationship between these two ligaments. Knowledge of the length and width of the native intra-articular ACL could play an important role in choosing the type of graft, as well as in the preparation of the graft. The intra-articular length of the ACL is important as it will determine the graft length that is available for firm fixation in the bone tunnels.⁵ An incorrect graft can lead to knee instability, reduced range of motion, failure of implant fixation, or even osteoarthritis in the knee joint.^{2,8,11,15} The ability to determine the length of the native ACL in advance could facilitate more efficient pre-operative planning. The bundles of the ACL have also been the subject of many research articles and are also an important component that should be considered during ACL reconstruction.^{5,7,9,16,30,31}

As far as can be established, no cadaveric studies have yet been conducted on a South African sample to determine a standardised length of the ACL, or whether independent factors influence ACL length in a clinically significant manner, especially as far as reconstructive surgery of a torn ACL is concerned.

Chapter 3: Aims

3.1 Anterior cruciate ligament and patellar ligament morphology

Rationale:

The morphology of the damaged ACL needs to be known in order to pursue effective reconstruction. Being able to accurately predict the ACL length when independent factors of the individual are known, would allow the pre-operative estimation of the length of the native ACL that needs to be reconstructed. Independent factors include maximum femoral epicondylar width (FECW) and patient height.

Aims:

1. Determine the length and width of the ACL and the length of the PL in a South African cadaver sample.
2. Establish whether it is possible to accurately predict the ACL length when independent factors such as maximum FECW, PL length, ACL width and height of the individual are known.

3.2 Radiographic anatomy and clinical implication of the anterior cruciate ligament

Rationale:

The pre-operative estimation of the length of the graft required for ACL reconstruction would assist with effective ACL reconstructive surgery. Examination of pre-operative MRI scans of the knee and associated ligaments is the most accurate method to evaluate the ACL before surgery. Apart from the availability and financial cost of MRI scans, it also requires very special scanning techniques and knee positions in order to visualise the entire length of the ACL. If the FECW is a good predictor of ACL length (determined in Aim 3.1.2), can the ACL length be determined using radiographs instead of MRI scans? Using radiographs to determine ACL length will reduce the cost of additional and specialised imaging modalities. Radiographs are the first step for knee injury examination and are readily available.

Aims:

1. Determine the length of the ACL and PL on specialised MRI scans of a South African sample.
2. Establish whether ACL length can be predicted using the maximum FECW obtained from an MRI.
3. Determine whether the maximum FECW obtained from radiographs is also a good predictor of ACL length.
4. Compare the reliability of the formula developed for MRI scans to determine ACL length, to that of the formula for radiographs, for the same purpose.

3.3 Histological composition of the anterior cruciate ligament and patellar ligament

Rationale:

It is important to know the micro-anatomy of the ACL in order to understand the macro-anatomy. This study compared the histological composition of the ACL and the PL. Understanding the microscopic structure of both these ligaments is important because in the case of ACL rupture, the ligament is often replaced using the PL.

Aims:

1. Determine the cell number, cell density, and nuclear morphology of the fibroblast cells of the ACL and PL.
2. Note the presence and diameter of blood vessels.
3. Compare the histological analysis of the similarities and/or differences between the ACL and PL by using light microscopy.

Chapter 4: Materials and methods

4.1 Anterior cruciate ligament and patellar ligament morphology

Ninety-one formalin-fixed adult cadavers with a mean age of 60 years (range 21 – 96 years) were included in the study. There were 26 female and 65 male cadavers included. The sample consisted of 55 white and 36 black cadavers. The average height of the sample was 1.69m (range 1.51m - 1.87m). Both right and left knees were used. The knees of 25 cadavers were excluded because of previous knee surgery, the presence of knee pathology or damage to the relevant ligaments.

The cadavers were obtained from the Department of Anatomy at the University of Pretoria and the University of Limpopo, Medunsa Campus. Ethical clearance was obtained from the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria. All cadaveric material used in this study was handled in accordance with the requirements of the South African National Health Act, Act 61 of 2003. From the researched literature¹¹ it had been concluded that sex; age and weight differences do not influence the length of the ACL. These demographic features were not researched further, as they were not thought to have an influence on the study outcome.

The cadavers were placed in a supine position with the leg raised and the knee stabilised at 90° flexion. The PL was exposed by reflecting the skin and overlying soft tissue. The length of the PL was measured at its midpoint from the inferior pole of the patella (α) to the superior aspect of the tibial tuberosity (β) (Fig. 4.1).^{13,52} This measurement represented the maximum available length of the tendinous portion of the PL graft for ACL reconstruction. The bony landmarks could easily be palpated in order to determine the attachment sites of the PL.

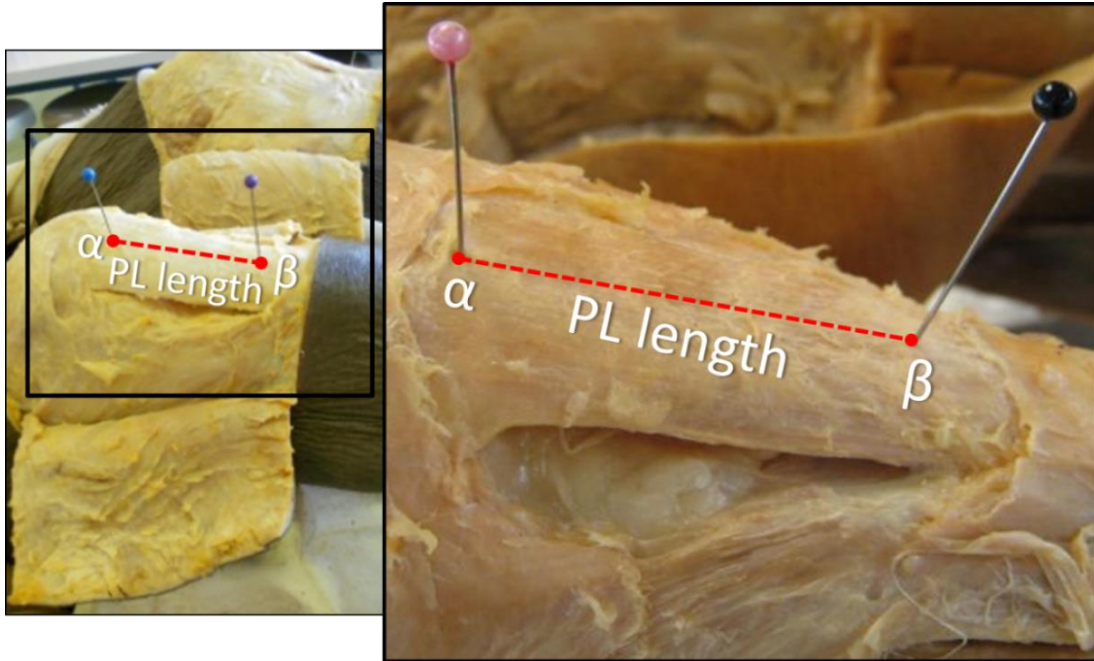


Fig. 4.1: Measurement of the PL length from the inferior pole of the patella (α) to the superior aspect of the tibial tuberosity (β).

After the PL length was measured, the ACL was revealed by transecting the attachment of the quadriceps femoris muscle to the patella. The patella and PL were reflected inferiorly (Fig. 4.2).

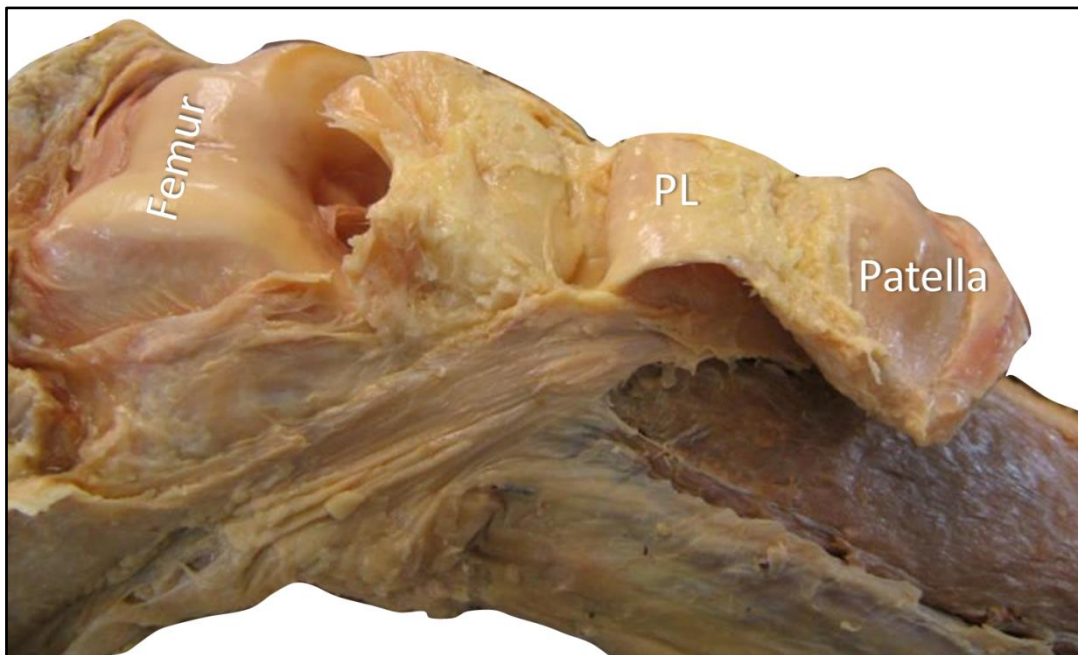


Fig. 4.2: Reflected PL to expose the knee joint and ACL.

The fat pad, deep to the PL, was removed in order to expose the ACL. *In situ* measurements (the width and length) of the ACL were then taken. The width was measured at the centre of the ligament (Fig. 4.3 a). The length of the ACL was measured from its femoral bony attachment to the tibial bony attachment (Fig. 4.3 b). The measurement of the ACL length was performed blindly by exploring the proximal attachment site with a probe until resistance from the femoral attachment site limited further progress. The length of the probe between the aforementioned femoral attachment site and the visible tibial attachment site was used to derive the length of the ACL. All measurements were taken with a mechanical dial-calliper (accuracy 0.01mm).

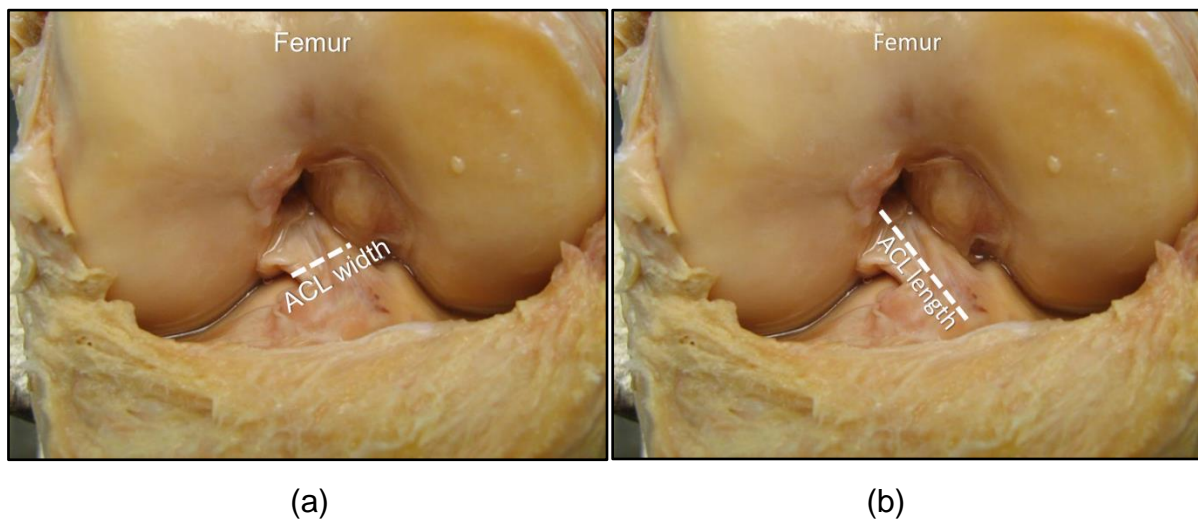


Fig. 4.3 a, b: Anterior view of exposed knee joint with ACL measurements.

In order to determine whether the ACL length could be accurately estimated, four independent variables were selected. A possible correlation between the ACL length (dependent variable) and the maximum FECW of the distal femur, height of the individual, PL length and ACL width (independent variables), were investigated. The height of the cadavers was recorded prior to being embalmed and was obtained from the cadaver records.

The soft tissue at the most medial and most lateral points of the femoral epicondyles was removed. The distance between these two points was measured (Fig. 4.4). PL length and ACL width were determined as part of the first aim of this study.

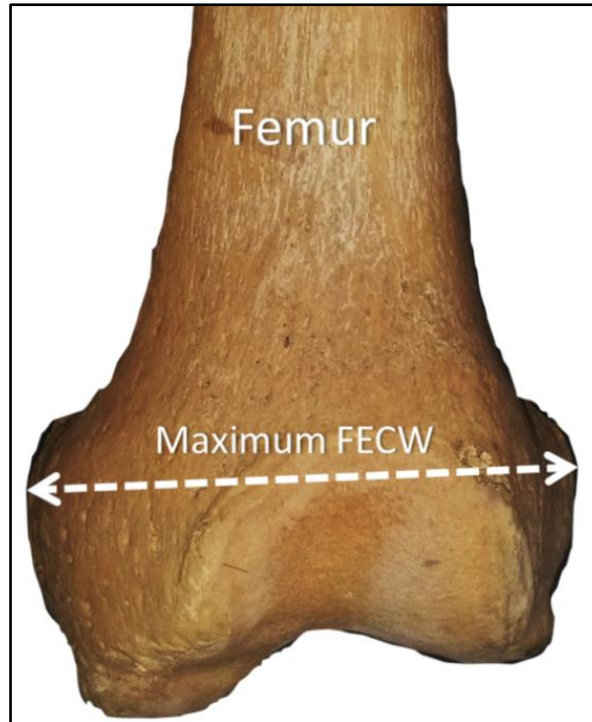


Fig. 4.4: The distal femur showing the measurement of maximum FECW.

Statistical analysis:

Boxplots were used to determine whether the data collected for each variable was symmetrically distributed. Simple descriptive statistics were conducted on all variables (mean, standard deviation (SD), minimum and maximum values). A paired t-test was performed to statistically evaluate the left and right sides. Statistical analyses were performed using the Pearson's correlation coefficient test to calculate associations between the dependent variable and each of the four independent variables. Finally, a linear regression model was developed using SAS 9.3 for Windows (Copyright © 2002-2010 by SAS Institute Inc., Cary, NC, USA), using ACL length as the dependent variable. Height, FECW, PL length and ACL width were the potential independent predictors.

4.2 Radiographic anatomy and clinical implication of the anterior cruciate ligament

Fifty-two patients who have previously undergone MRI and radiographic evaluation for non-specific knee pain, were used in the study. The sample had a mean age of 40 years (range 14 – 67 years), of which 30 were male and 22 female patients. Patients with signs of trauma or degenerative arthritis were excluded from the study. Eight outliers were excluded from the sample, leaving a total of 44 patients included in the study.

The MRI scans and radiographs were sourced from the database of the Little Company of Mary Medical Centre (Capital Radiology), Pretoria. All imaging material were examined with the assistance of two consultant radiologists. The entire length of the ACL needed to be visible on the MRI, as well as the maximum FECW on both the MRI and radiograph of each patient. The radiographs and MRI scans of each patient were taken within a six month period from one another. Ethical clearance was obtained from the Research Ethics Committee, Faculty of Health Sciences, University of Pretoria. Permission to conduct research and examine patient records retrospectively has been obtained from the appropriate hospital authority.

All images were obtained with the patient in the supine position with the knee slightly flexed and positioned feet first into the MRI scanner. The inferior pole of the patella was in the middle of the coil. Sponges and sandbags were used to immobilise the knee. The ACL runs obliquely within the knee joint and in order to view the entire length of the ACL, the angles for scanning the knee were as follows:

- Transverse: Angle parallel to the tibial plateau on the sagittal image and parallel to the menisci on the coronal image.
- Coronal: Angle parallel to the femoral condyles on the transverse image and perpendicular to slightly oblique ($\pm 5^\circ$ anterior) on the tibial plateau on the sagittal image.
- Sagittal: Angle perpendicular to the tibia on the coronal image and parallel to slightly oblique ($\pm 5^\circ$ medially) on the medial aspect of the lateral femoral condyle.

Two MRI scanners were used. Some patients were scanned using a Philips 3T Ingenia MRI scanner. A 16 channel dedicated knee coil was employed. The standard sequences were T2 or Proton Density Fat Suppression in three anatomical planes (transverse, sagittal and coronal) and two Proton Density sequences (sagittal and coronal). The sequences were T2 Fat Saturated (TR 2800 - 4000/TE 50ms), and Proton Density (TR 2600 – 4500/TE 25ms) with 90° flip angles. The slice thickness was 3mm with a gap of 10%. The direction of the sagittal scans were feet to head with 65 superior and 65 inferior oversampling, 30 slices. The field of view (FOV) from anterior to posterior (AP) was 140mm, feet to head (FH) was 170mm and from right to left (RL) was 90mm. The transverse scan direction from feet to head was with 65 superior and 65 inferior oversampling, 35 slices. FOV AP 150mm, RL 170mm and FH 110mm. Lastly, the coronal scan direction from right to left was with 10 right and 10 left oversampling, 30 slices. FOV AP 100mm, RL 130mm and FH 140mm. Measurements were performed on the DICOM files using the manufacture's software (GEARView Basic 2.1 PACSGEAR™ 2013 USA). The remaining patients were scanned using a Siemens 1.5T Symphony Tim MRI scanner. An eight channel dedicated knee coil was employed. The standard sequences were Proton Density with Fat Suppression in three anatomical planes (transverse, coronal and sagittal) and two Proton Density sequences (sagittal and coronal). The sequences were Proton Density Fat Saturated (TR 3000/TE 31ms), and Proton Density (TR 2251/TE 32ms) with 150° flip angles. The slice thickness was 3mm with a gap of 10%. The sagittal scan direction was from anterior to posterior with 70 oversampling, 27 slices. FOV 180mm and FOV phase 80mm. Coronal scan direction from right to left with 40 oversampling, 25 slices. FOV 180mm and FOV phase 100mm. Transverse scan direction from right to left with 80 oversampling, 29 slices. FOV 180mm and FOV phase 80mm.

The aforementioned knee position and angles allowed complete visualisation of the ACL throughout its entire length on the specialised sagittal MRI section (Fig. 4.5). The scans were done in the true sagittal plane, but occasionally a minimal obliquity was used. The length of the ACL was measured from its attachment on the tibia to its femoral attachment site. The measurement was taken at the level where both attachment sites of the ACL were visible on the MRI using the on-screen standard DICOM analysis program calibrated for each image.



Fig. 4.5: Sagittal MRI section of the knee indicating measurement of the ACL length.

The length of both the PL and the ACL was measured on the same sagittal MRI section. The maximum PL length was measured from the inferior pole of the patella to the superior portion of the tibial tuberosity (Fig. 4.6). This measurement represented the length of the PL graft used for ACL reconstruction.

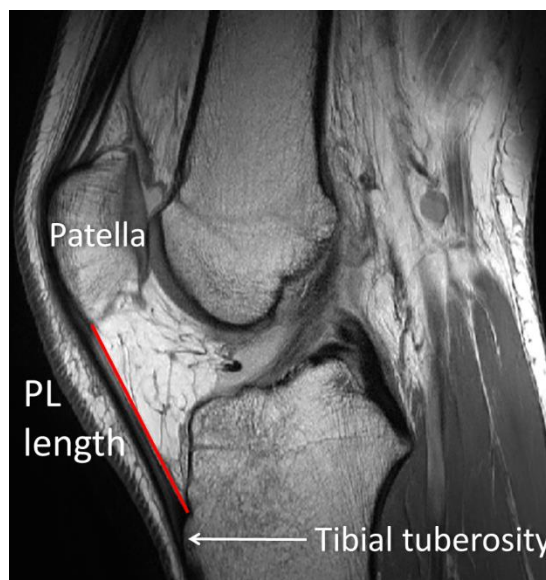


Fig. 4.6: Sagittal MRI section of the knee indicating measurement of the PL length.

The coronal MRI section was used to measure maximum FECW. This measurement was taken at the widest portion of the femoral epicondyles at the level of the MRI section where the meniscal bodies and PCL insertion were visible (Fig. 4.7).

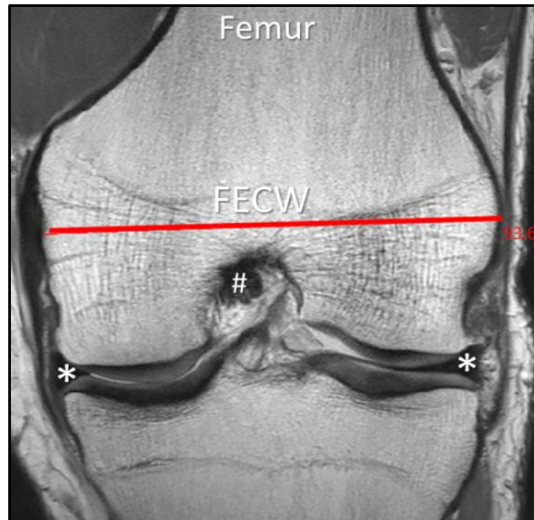


Fig. 4.7: Coronal MRI section of the knee at the level where the meniscal bodies (*) and posterior cruciate ligament (#) are visible. The measurement of the maximum FECW is indicated by the red line.

The maximum FECW was measured at the widest point on the radiograph of the same patient (Fig. 4.8). All radiographs were taken in the standard frontal plane.

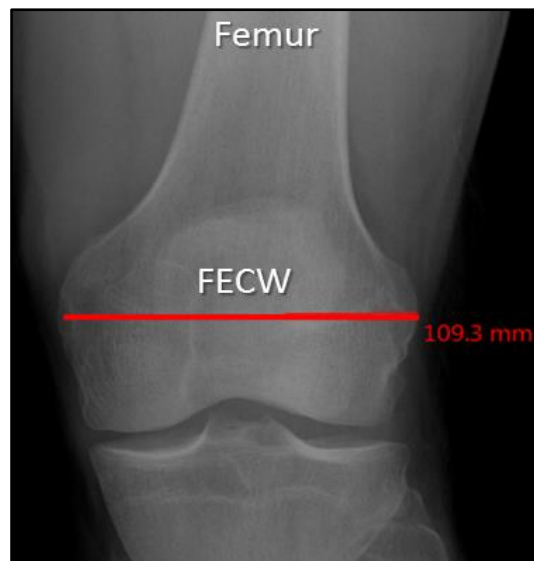


Fig. 4.8: Anteroposterior radiograph of the knee. The measurement of the maximum FECW is indicated.

In order to determine whether the ACL length could be accurately estimated, two potential predictors were selected. A possible correlation was investigated between the ACL length (dependent variable) and the maximum FECW of the distal femur and PL length (independent variables) on the MRI scans. Unfortunately, the height of the patients was not known and could not be included as a possible independent variable. The maximum FECW obtained from both the MRI and radiograph images of each patient were compared. It was established that ACL length could be accurately determined using the maximum FECW. This comparison between the FECW measurements would determine whether an MRI or radiograph is the best predictor of ACL length.

Statistical analysis:

Boxplots were used to confirm that the data collected for each variable was symmetrically distributed. Simple descriptive statistics were conducted. A paired t-test was performed to test for differences in the FECW values measured on the MRI and radiograph images. Pearson's correlation coefficients were calculated to test for pairwise associations between the dependent variable and the three independent variables. Finally, two separate linear regression models were developed using SAS 9.3 for Windows (Copyright © 2002-2010 by SAS Institute Inc., Cary, NC, USA) with ACL length as the dependent variable, together with three independent variables, namely the PL length, the FECW measured on an MRI and the FECW measured on a radiograph.

4.3 Histological composition of the anterior cruciate ligament and patellar ligament

The ligaments of ten unfixed cadaveric donors were procured from the National Tissue Bank, University of Pretoria. Using clean surgical techniques, 18 ACLs and 10 PLs were harvested. Both the ACL and PL of the same donor were harvested wherever possible. The cadavers had a mean age of 50 years (range 28 – 67 years). Three males and seven females were included in the study sample.

Permission to conduct research at the National Tissue Bank, University of Pretoria was obtained from the Head of the National Tissue Bank. All cadaveric material used was handled in accordance with the requirements of the South African National Health Act, Act 61 of 2003. Written informed consent was obtained from the next of kin, life partner and/or legal individual of the deceased donor. Each donor was inspected for any clinical signs and symptoms of infection, liver disease, transmittable diseases and visible tears or trauma to the ACL. Serological (blood) samples were collected from the donor and tested for the presence of HIV, Hepatitis B and C, as well as syphilis. Where the donor tested positive for any of the aforementioned conditions, or showed any clinical sign of disease or trauma to the ACL, the donor was excluded from the study. Donors who did not show any physical signs of disease and/or trauma to the ACL and with negative serological test results were included in the study. The serological testing was not conducted specially for this study, but is routine practice at the National Tissue Bank.

This component of the study was unfortunately limited by sample size due to the speciality of the samples. The sample sizes were calculated according to the number of donors available for the study and was discussed with, and approved by the statisticians.

The donors were placed in a supine position with the knee stabilised at 90° flexion. A vertical incision was made over the knee joint. The skin and overlying soft tissue were reflected to expose the PL. The middle one third of the PL was removed from the most inferior pole of the patella to the superior aspect of the tibial tuberosity. The patella and remaining PL were reflected. The fat pad, filling the space between the PL and ACL, was removed to expose the ACL. The knee joint was hyperflexed in order to gain access to the ACL. The ACL was dissected loose from its origin and insertions sites (Fig. 4.9). The ACL and PL were placed and fixated in sterile, labelled, screw capped bottles containing 2.5% gluteraldehyde (GA) / formaldehyde (FA) fixative (1ml of 25% GA, 1ml of 25% FA, 5ml phosphate (PO₄) buffer (0.15M) and 3ml of double distilled water (ddH₂O)). The procured tissue was preserved in the quarantine fridge at 4°C until the serological tests were received. Negative blood test results allowed the release of the tissue for histological processing.



Fig. 4.9: ACL removed from donor (with indicated regions).

The synovial tissue surrounding the ACL was carefully removed using blunt forceps. Cross-sections were then made at the central part of the ligament (Fig. 4.10).

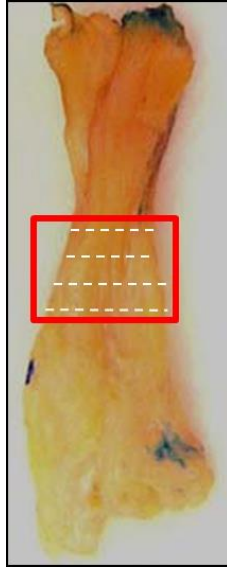


Fig. 4.10: The central region of the ACL. The white dotted lines indicate the areas where cross-sections were made.

The tissue was kept in the fixative for a maximum of 48 - 72 hours, where after it was rinsed three times in 0.075M PO₄ buffer (pH = 7.4) for 15 minutes each, before the tissue samples were dehydrated in 30%, 50%, 70%, 90% and three changes of 100% ethanol. The tissue samples were left in 100% ethanol overnight. The next day the tissue samples were placed in 50% xylene, then in ethanol for 30 minutes and finally in 100% xylene for two hours. The tissue samples were placed in a 30% wax: 70% xylene solution for an hour at 60°C, followed by a 70% wax: 30% xylene solution for an hour. The samples were then transferred to a 100% wax solution for two hours at 60°C. In the final step of embedding, the tissue was placed in a steel mould filled with wax and covered with a marked grid. The moulds with the grids were then placed on a cooling plate to allow the wax to cool and set. Sections of 4µm were made using a Leica RM 2255 wax microtome, where after the sections were placed on glass slides. The slides were stained with haematoxylin and eosin (H&E).

The H&E staining was achieved by firstly clearing the section slides using xylene for ten minutes to remove the paraffin wax. The slides were then placed in a series of descending ethanol concentrations to rehydrate the tissue. The series started with placing the slides twice in 100% ethanol for two minutes, then in 90% ethanol for a minute, followed by 70% ethanol for another minute.

Before the slides were placed in the haematoxylin for five minutes, they were rinsed in ddH₂O for a minute. After the haematoxylin staining, the slides were rinsed with tap water and placed into the Scott's blue buffer solution for five minutes. The tissue was then dipped in acidic ethanol, where after it was counterstained by leaving it in eosin for five minutes. After the eosin step, the tissue was dipped in a series of ascending ethanol concentrations (70%, 90% and 100% ethanol) to dehydrate the tissue. This ensured that the excess dye was removed. Lastly, the slides were dipped in xylene before the cover slip was mounted using entellen. The slides were viewed with a Nikon Optiphod transmitted light microscope and the histological composition of the ACL and PL was determined.

Two undamaged areas were determined using a low magnification (10x objective magnification) for each sample. The selected areas were then further magnified (40x objective magnification) in order to count the number of fibroblasts present. The area analysed was determined using Image Tool Version 3.0 (Copyright © 1996-2002 by UTHSCSA). The cell density was determined by dividing the total number of cells by the area analysed. The morphology of the fibroblast nuclei were measured under 100x magnification and classified as either spheroid (< 5µm) ovoid (5 - 10µm) or fusiform (> 10µm). Slides on which blood vessels were noted, were photographed. The diameter of the blood vessels was also measured.

The histological composition of the ACL and PL was then compared in order to determine the similarities and/or differences between the ACL and the PL graft used to replace a torn ACL.

Statistical analysis:

Simple descriptive statistics were conducted on all variables (cell number, cell density and nuclear diameter). Paired t-tests were performed to compare the values found between the ACL and PL. The diameter of the fibroblast nuclei were measured to classify them as either ovoid, spheroid or fusiform. The frequency distribution of each group was then determined. The characteristics of both the ACL and PL were compared.

Chapter 5: Results

5.1 Anterior cruciate ligament and patellar ligament morphology

All data were symmetrically distributed. The descriptive analysis results are shown in Table 5.1. The paired t-test revealed a statistically significant difference between the length of the right and left ACLs ($p < 0.001$) of an individual. The ACL width, PL length and FECW of the right and left knees of each individual showed to be similar ($p > 0.05$). Owing to the differences found in ACL length, the measurements for right and left are summarised separately in Table 5.1.

Table 5.1: Simple descriptive statistics of the length and width measurements of the ACL and PL of the cadaver sample ($n = 91$).

	Right			Left		
	ACL length (mm)	ACL width (mm)	PL length (mm)	ACL length (mm)	ACL width (mm)	PL length (mm)
Mean	32.44	8.23	45.66	33.71	7.92	45.54
SD	4.06	1.96	5.71	4.56	1.58	5.42
Minimum	22.90	5.10	31.56	24.20	4.16	32.02
Maximum	39.90	13.74	59.17	45.14	12.62	58.15

The result of the Pearson's correlation coefficient test can be seen in Table 5.2. The r-value indicates which of the four independent variables demonstrated a significant positive or negative linear relationship to ACL length (dependent variable). An r-value of 0.30-0.39 indicates a moderate positive correlation, while an r-value greater than 0.40 indicates a strong positive correlation. Where the p-value of the Pearson's correlation coefficient test is $p < 0.05$, it is considered as a statistically significant correlation.

Table 5.2: Correlation matrix of cadaveric component.

	ACL length	
	Right	Left
Height	$p = 0.01^a$	$p = 0.12$
	$r = 0.31$	$r = 0.19$
PL length	$p = 0.92$	$p = 0.96$
	$r = 0.01$	$r = 0.01$
FECW	$p = 0.00^a$	$p = 0.04^a$
	$r = 0.36$	$r = 0.25$
ACL width	$p = 0.26$	$p = 0.39$
	$r = 0.14$	$r = 0.11$
^a Statistically significant correlation		

Statistical inference followed after the completion of the descriptive statistics in order to predict ACL length. By means of backward elimination, using a multiple regression model, it was observed that both height and FECW had a statistically significant correlation to ACL length on the right side. However, FECW was a more reliable predictor for estimating ACL length than height ($r = 0.36$ vs. $r = 0.31$ respectively). For the left side, FECW was the only independent variable with a statistically significant correlation to ACL length ($p = 0.04$). Based on these evaluations, no additional variables were used to compile an equation for pre-operative ligament length estimation. PL length, height and ACL width were therefore discarded as possible predictors of ACL length. A linear regression formula was developed for predicting ACL length for both right and left knees and, therefore, optimum graft length to re-establish the intra-articular length of the torn ACL. The following equations could be used to calculate ACL length if the maximum FECW is known (Table 5.3):

Table 5.3: Equations to predict ACL length for either a left or right knee.

Right	Left
ACL length = $0.25 (\text{FECW}) + 11.18$	ACL length = $0.19 (\text{FECW}) + 17.28$.

5.2 Radiographic anatomy and clinical implication of the anterior cruciate ligament

Simple descriptive statistics of the ACL and PL lengths on specialised MRI scans were conducted (Table 5.4). The boxplots confirmed that the data were distributed symmetrically.

Table 5.4: Simple descriptive statistics of radiographic length measurements.

	ACL length (mm)	PL length (mm)
Mean	40.36	51.50
SD	3.46	6.08
Minimum	32.50	39.10
Maximum	48.17	65.80

The paired t-test revealed a significant difference between the mean MRI and radiograph measurements for FECW ($p < 0.0001$). The Pearson's correlation coefficient was determined with ACL length as the dependent variable for both MRI and radiograph, with the independent variables being PL length and FECW on MRI, and FECW on radiograph (Table 5.5).

Table 5.5: Correlation matrix of radiographic component.

	ACL length
PL length	$p = 0.01^a$
	$r = 0.37$
FECW on MRI	$p < 0.0001^a$
	$r = 0.88$
FECW on radiograph	$p < 0.0001^a$
	$r = 0.83$
^a Statistically significant correlation	

It was established that the FECW measured on both the MRI and radiograph were sufficient to predict ACL length. The coefficient of determination (R^2) was established to measure how closely the regression line approximates the real data points. The values were determined to be MRI: $R^2 = 0.77$ and Radiograph: $R^2 = 0.69$. Both these values fall within acceptable range. However, it is clear that MRI is the more accurate predictor of ACL length, although a radiograph is a good alternative, despite the slightly lower R^2 - value. Two linear regression models were developed to predict ACL length according to the width of the femoral epicondyles measured on either an MRI or a radiograph (Table 5.6).

Table 5.6: Equations to predict ACL length by means of FECW for either MRI or radiograph.

MRI	Radiograph
ACL length = $0.47(\text{FECW}) + 2.28$	ACL length = $0.31(\text{FECW}) + 11.31$

5.3 Histological composition of the anterior cruciate ligament and patellar ligament

Simple descriptive statistics were conducted on the cell number and cell density of the ACL and PL (Table 5.7).

Table 5.7: Simple descriptive statistics of the ACL and PL histology.

	Cell number		Cell density (cells per μm^2)	
	ACL	PL	ACL	PL
Mean	10.45	10.54	2832.85	3326.12
SD	4.42	6.33	1132.48	1724.95
Minimum	4.50	4.50	1038.40	987.20
Maximum	19.50	28.50	5586.78	7231.14

The paired t-tests revealed that no statistically significant difference ($p > 0.05$) existed between the ACL and PL, for both cell number ($p = 0.78$) and cell density ($p = 0.13$) (Fig. 5.1). However, the PL indicated a slightly higher cell density than the ACL.

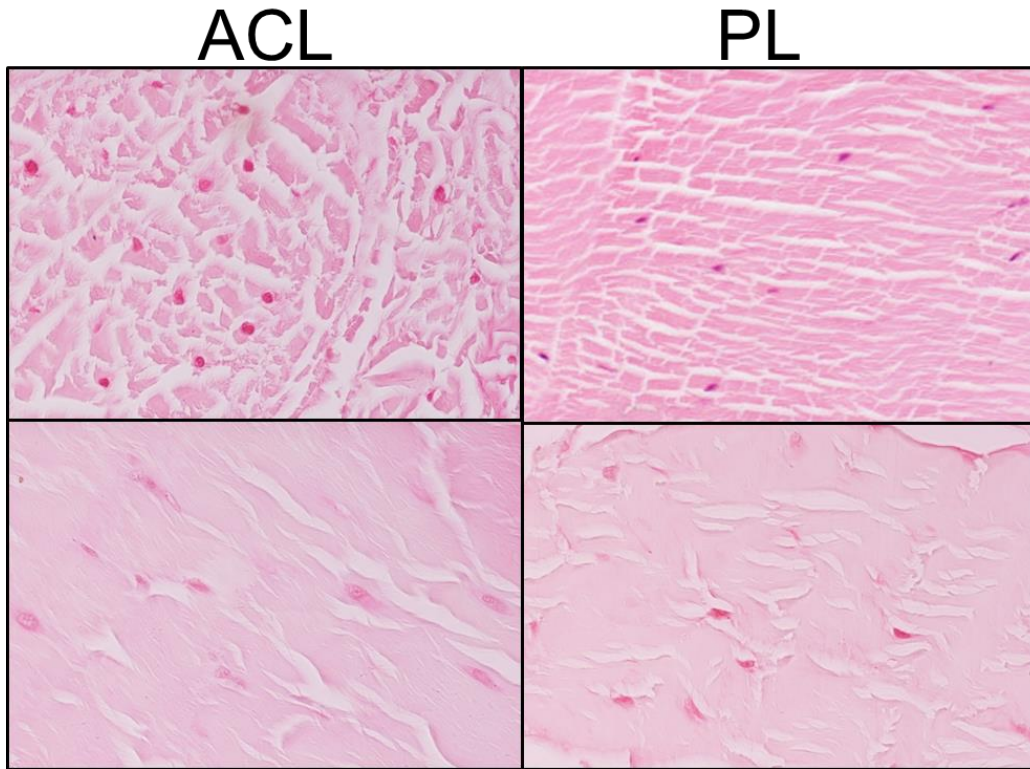


Fig. 5.1: Cell number of the ACL and PL

The nuclear morphology of the fibroblasts was calculated according to their percentage of occurrence in both the ACL and PL (Table 5.8) and (Fig. 5.2).

Table 5.8: The frequency distribution of the nuclear morphology of the fibroblasts of the ACL and PL.

	Nuclear morphology (%)		
	Spheroid (< 5 μ m)	Ovoid (5 - 10 μ m)	Fusiform (> 10 μ m)
ACL	35.56	54.44	10.00
PL	41.27	28.57	30.16

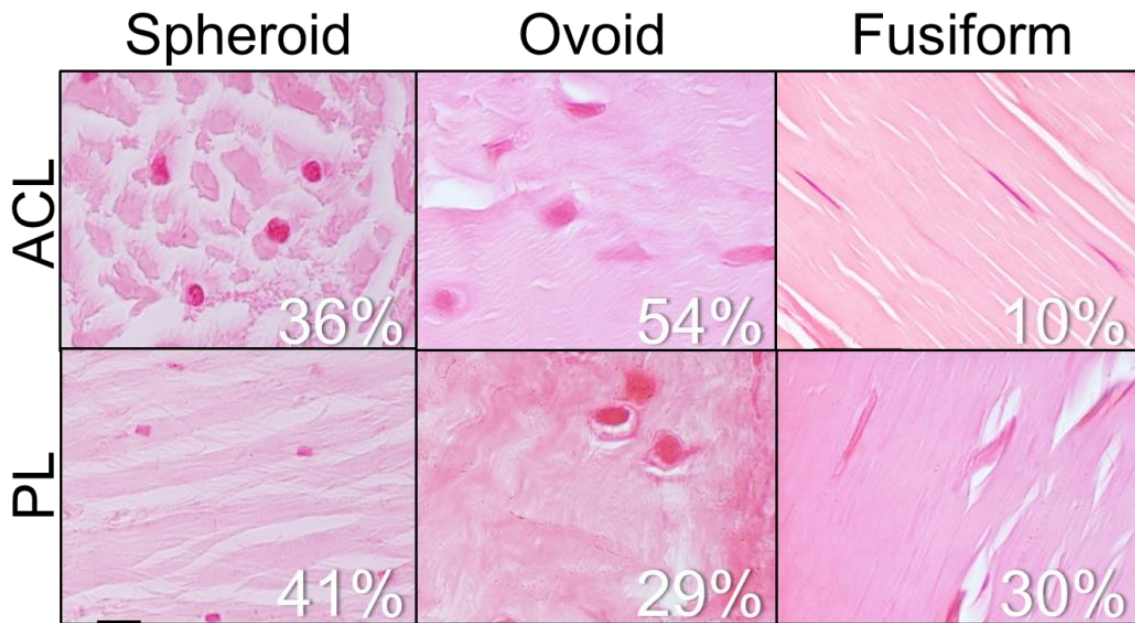


Fig. 5.2: Fibroblast nuclei morphology of the ACL and PL

The most abundant fibroblast nuclei found within the central part of the PL was spheroidal shaped nuclei (41%). The ACL fibroblasts mainly consisted of ovoid shaped nuclei (54%).

The presence of blood vessels on the sections of the ACL and PL were noted. Blood vessels were present in 14 out of the 17 areas examined (82%) in the ACL samples, and in 7 out of the 13 areas examined (54%) in the PL samples. Varying blood vessel diameters were noted, indicating the presence of arterioles, pre-capillaries, capillaries and venules in the ACL and PL. These vascular bundles were located in the loose connective tissue, between the collagen fibre bundles of the ligaments (Fig. 5.3).

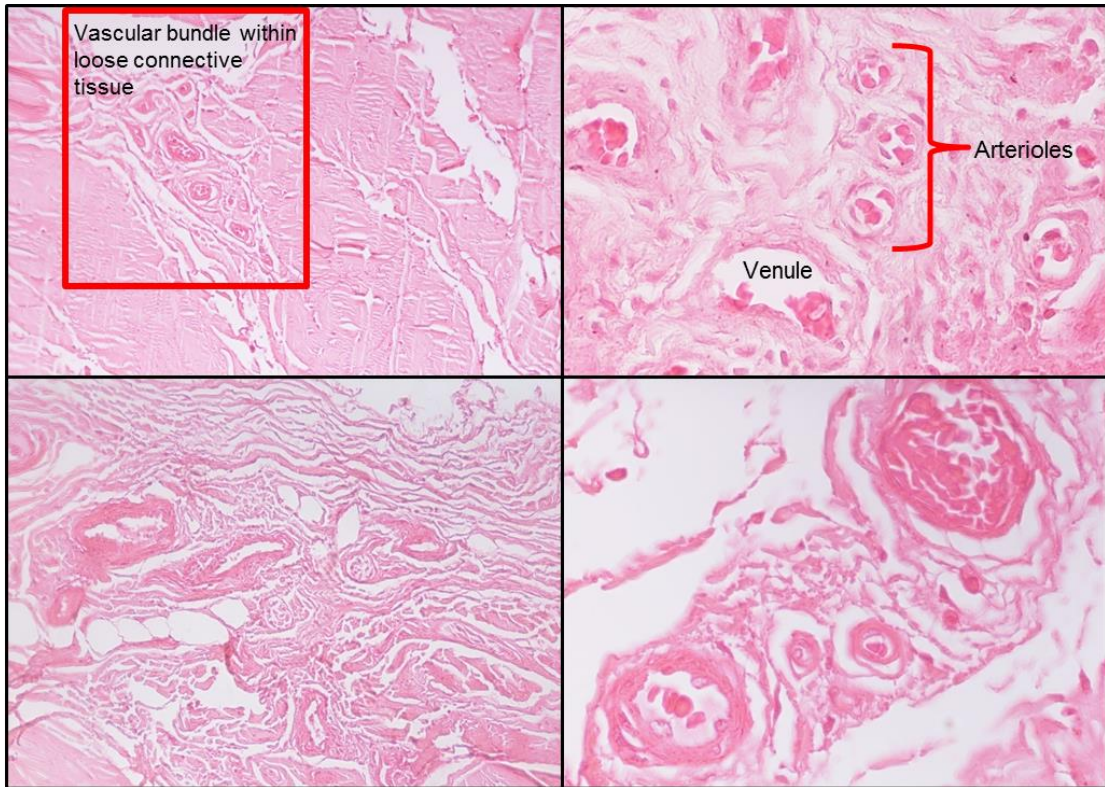


Fig. 5.3: Blood vessels with varying diameters within the loose connective tissue of the ligaments.

Chapter 6: Discussion

The ACL is one of the most frequently injured knee ligaments. ACL reconstructive surgery is currently the only method of ensuring knee stability after an ACL tear has occurred. Of all the possible grafts that could be selected to replace the torn ACL, the BTB graft has shown to be one of the best replacement grafts. Successful graft reconstruction requires knowledge of the morphological characteristics of the ACL and BTB graft at macroscopic and histological levels to ensure the normal anatomy is restored. Unfortunately, various complications could occur. In order to prevent or reduce complications, proper pre-operative planning is essential. MRI is currently the imaging modality of choice to evaluate ACL injuries, but is not readily available to all South African citizens. It would therefore be beneficial if an alternative method could assist in the pre-operative planning for ACL reconstruction. Graft mismatch could be minimised if the correct ACL length could be accurately predicted pre-operatively. This would also allow the surgeon to determine the correct graft length needed to restore normal ACL biomechanics.

6.1 Anterior cruciate ligament and patellar ligament morphology

ACL length:

The first aim of this study was to determine the morphology of the ACL and PL. It is usually expected that bilateral ligaments will show similar morphology. None of the literature reviewed for this study differentiates between left and right knees. Jamison *et al.*⁴³ reported that the total length and cross-sectional area of the ACL is similar between right and left knees. However, Scanlan *et al.*⁴⁹ reported small side-to-side differences between the injured and contralateral knee. In the cadaveric component of this study it was found that the left and right ACL lengths differed statistically. Because of this difference, all subsequent measurements of the ACL width and PL length were analysed separately for left and right knees.

This study shows that the mean intra-articular ACL length of the right knees was 32.4mm, with a range between 22.9mm - 39.9mm. The left ACL lengths were found to be 33.7mm, with the values ranging from 24.2mm - 45.1mm. The radiographic component of this study reported slightly different values than the cadaveric component. Mean ACL length was 40.4mm and ranged between 32.5 and 48.2mm. The literature had shown the mean length of the ACL to be 20mm⁵⁸, 31mm³⁴, 32mm^{5,9,20,22,30}, 38mm^{8,29} and 39mm⁵⁹. The ACL length exhibited some variability, with ranges of 20mm - 25mm¹¹, 22mm - 41mm^{5,9,20,22,30}, 25mm - 35mm³⁴, 31mm - 38mm¹⁶ and 37mm - 41mm⁵⁹ reported previously. Both the left and right ACL lengths of the cadaveric component fell well into the acceptable range when compared to the results of previous studies. On the other hand, the radiographic component values obtained in this study were higher than those found in the literature. It should be noted that the cadaveric ACLs were measured from anterior to posterior, while the radiographic ACLs were viewed on a sagittal image.

ACL width:

The width of the right ACL had a mean value of 8.2mm, while the width of the left ACL had a mean value of 7.9mm. The right ACL width ranged from 5.1mm - 13.7mm, and the left from 4.2mm - 12.6mm, which differed slightly from the range of 7 - 12mm determined by various authors.^{5,7,30,34,59} Beasley *et al.*¹⁶ found ACL width to be 10mm – 12mm. The ACL was reported to have a mean width of 10mm³⁴ and 11mm⁸. Previous literature reported mean ACL width values that were greater than those in this study. It is important to keep in mind that the width of the ligament was taken at its narrowest point, which could account for the lower results found in the cadaver sample. It is vital to ensure that the graft width mirrors that of the native ACL being replaced. This will restore the morphological characteristics of the ligament and prevent future complications.

PL length:

Using the PL as a BTB graft to replace an injured ACL has developed into a successful procedure to restore normal knee kinematics. BTB graft size mismatch is a potential pitfall when performing ACL reconstructive surgery. Knowledge of the available length of the PL is important in order to ensure proper graft fixation, as a graft that is too long would protrude from the bone tunnel and compromise graft incorporation.¹¹

The PL mean length of this sample was found to be 45.7mm and 45.5mm for the right and left cadaveric PLs respectively. Measurements on the right side ranged between 31.6mm - 59.2mm and on the left between 32.0mm - 58.2mm for the cadaveric component. The radiographic component of this study determined the mean PL length to be 51.5mm with a range from 39.1mm - 65.8mm. The cadaveric measurements were found to be similar to the results from previous studies. Brown *et al.*¹¹ recorded a range of 45mm - 50mm and Denti *et al.*⁵⁸ a mean length of 45.5mm. The radiographic component on live patients reported a slightly higher mean PL length value, but the range fell within agreeable values. On average, the PL was 14mm longer than the ACL, and therefore was sufficient in length to allow effective fixation of the graft. Variation in PL length could explain the occurrence of graft mismatch found with ACL reconstructive surgery. In cases where the required BTB graft length is not available to replace the ACL, other grafts such as the HS graft should be considered.

Limitations:

Dissection of the cadaveric ACL was challenging, due to the diagonal course of the ligament within the knee joint. The measurement was therefore taken blindly, which could lead to inaccurate measurements of the true ACL length. The variability observed between left and right ACL length could be due to the dexterity (right handed) of the observer and the difficulty to measure this ligament. The dominant leg of the patient may also have an influence, but unfortunately this information is not available in cadaver records. The slight differences between the measurements from this sample and other reported measurements could be attributed to the respective

population differences. The measuring technique used could also account for the dissimilarities found among the studies referred to by Brown *et al.*¹¹ and Denti *et al.*⁵⁸ The higher values observed in the radiographic components could be owed to the precision of MRI scans, which allow for the exact measurement of ligament lengths, as the attachment sites were clearly visible, which is not the case in cadaveric dissections. This could have resulted in an overestimation of ligament lengths. The level of the sagittal MRI section at which the measurements were taken could also influence the visible ligament length measured.

6.2 Best predictor of anterior cruciate ligament length

Several previous studies have expressed the need for using independent factors, such as height, weight and sex of a population to determine intra-articular ACL length.^{11, 42, 51, 58} In the cadaveric component of this study the ACL length and PL length demonstrated no statistically significant correlation with each other. This confirmed the findings by Denti *et al.*⁵⁸ No significant correlation appeared between ACL length and ACL width for either knee. The significant correlation between ACL length and height ($p = 0.01$ and $r = 0.31$) on the right side was not unexpected. Brown *et al.*¹¹ found a strong correlation on a large MRI study sample consisting of 414 knees. They concluded that patient height could predict the required length of the BTB graft used for ACL reconstruction. In contrast, Denti *et al.*⁵⁸, used 50 reconstructed and 9 cadaver knees, but were unable to confirm the relationship between ACL length and patient height.

An extensive literature review revealed no previous studies that used FECW as a possible predictor of ACL length. The hypothesis of this study was that the size of the distal femur would directly determine the size of the ACL. The distance between the two femoral epicondyles can easily be palpated during physical examination, and is clearly visible on radiographic imaging, both on radiographs or coronal MRI scans. FECW can therefore easily be measured and as the femur directly influences the height of a patient it was thought to be a good possible predictor of ACL length. The Pearson's correlation test confirmed the hypothesis and showed that ACL length had

a significant correlation with FECW on the right ($p < 0.01$) and on the left ($p = 0.04$) knees. It was also statistically determined that FECW could account for the other three independent variables (height, PL length and ACL width) in predicting ACL length, as FECW had a strong correlation with all other variables. FECW was thus found to be the most reliable predictor of ACL length. A statistical model was developed for the estimation of ACL length when FECW is known. The R^2 -value of 0.13 indicated that 13% of variation found in right ACL lengths (among the cadavers) could be accounted for by the equation. On the left, the equation could account for 6% in sample variation ($R^2 = 0.06$). Therefore, measuring the FECW of the patient might allow for pre-operative estimation of the length of the ACL of that patient, by using the previously mentioned linear regression formulas (Table 5.3). These values could then be used to predict the original length of the injured ACL.

Maximum FECW was used in the current study to explore a possible correlation with ACL length. The positive, but weak correlation observed between the ACL length and maximum FECW in the cadaver sample could be explained by remnants of soft tissue covering the epicondyles after dissection, which may have led to inaccurate measurement of the maximum FECW. Patient height is often unknown and FECW is a practical measurement as it is commonly available on radiograph and MRI. In this sample, FECW proved to be a more reliable predictor than height for ACL length estimation. When comparing ACL length and FECW, a definite, positive correlation between the measurements ($p < 0.05$) was noticed. However, the low R^2 -values of the linear regressions indicated that in a South African cadaver sample, the FECW cannot be the sole predictor of ACL length. Pre-operative mismatch of the graft length could occur if the FECW is measured without the incorporation of additional imaging modalities. Intra-operative measurements may also assist to determine ACL length, and therefore the required graft length.

6.3 MRI versus radiograph for anterior cruciate ligament length determination

On account of the findings of the cadaveric component of this study, namely that FECW proved to be a more reliable predictor than height, patient height was not considered as an independent variable in the radiographic component. Maximum FECW was used instead, since it was found to be the strongest predictor of ACL length in the cadaver sample. The radiographic component revealed a statistically significant difference between the mean MRI and radiograph measurements for maximum FECW. This indicated the formulation of two separate linear regression models (equations). The Pearson's correlation coefficients revealed that all three variables (PL length, FECW on MRI and FECW on radiograph) demonstrated significant positive linear relationships with ACL length. PL length showed the least significant correlation with ACL length and was therefore discarded as a predictor of ACL length. The maximum FECW measured on both MRI and radiograph revealed strong positive correlations with ACL length ($p < 0.0001$). This was much more accurate than the cadaver sample. It was therefore established that the FECW measured on both MRI and radiograph were sufficient predictors of ACL length. This confirmed the findings of the cadaveric component of this study that FECW is the best predictor of ACL length.

The FECW measured on MRI showed the best r -value ($r = 0.88$) when compared to FECW on radiograph ($r = 0.83$). This finding was further confirmed by the coefficient of determination (R^2). The R^2 -value of the maximum FECW measured on MRI was 0.77 which implied that the equation could account for 77% of the variations found in ACL length. This proves that determining the FECW on a coronal MRI is more accurate and therefore was the best predictor for estimating ACL length. The FECW measured on a radiograph had an R^2 -value of 0.69 which was slightly lower than the R^2 -value of the MRI sample, but still fell within the range of a good predictor of ACL length. Radiographs could therefore be used to predict ACL length in cases where MRI scans are unavailable. MRI scanners are often not available to patients due to the cost related to this imaging modality, whereas radiographs are readily available

and inexpensive when compared to MRI scans. It would be beneficial to be able to use radiographs instead of MRI scans to assist with the pre-operative planning for ACL reconstructive surgery. Two separate equations were developed in order to predict ACL length pre-operatively by using the maximum FECW measured on either an MRI or radiograph (Table 5.6). It would be useful to predict graft size pre-operatively to prevent inadequate graft harvesting. Once the ACL length has been determined by using the FECW measured on either a radiograph or MRI, Brown *et al.*¹¹ concluded that 10mm should be added in order to obtain the ideal tendinous portion of the ligament graft, making the BTB graft ideal, as this study showed that on average, the PL is 14mm longer than the ACL. The additional length should be taken into account prior to graft harvesting.

The fact that the ACL courses diagonally through the knee joint may have caused difficulties in visualising the entire length of the ACL. In those cases, the MRI needed to be slightly adjusted by using a minimal obliquity which can only be done by some specialised MRI machines. MRI scanners are not always freely available to all patients, as all hospitals cannot afford this expensive equipment and those who do, are overbooked. In addition to this, not all patients have the funds to afford this imaging modality. The patients used for this component of the study all had some form of knee pathology and they were therefore not healthy subjects. However, the radiologists thoroughly examined the scans to exclude patients with compromised ACLs from the study.

6.4 Histological composition of the anterior cruciate ligament and patellar ligament

Fibre orientation:

In line with the findings of Zhu *et al.*³⁷, it was observed that both the ACL and PL are composed of closely packed collagen bundles. It could be expected that the ACL and PL would have structural differences, since their primary functions differ. The ACL needs to withstand stresses in multi-axial planes with various tensile strains.^{20,35,37} The H&E stained PL tissue showed a homogenous arrangement of parallel collagen fibres with a smooth and wavy appearance. On the other hand, the ACL had various collagen fibre arrangements. Parallel arranged fibres were observed which was similar to the PL, but some areas displayed perpendicular fibres crossing each other (Fig. 6.1). These findings were confirmed by various previous authors.^{20,25,35,37}

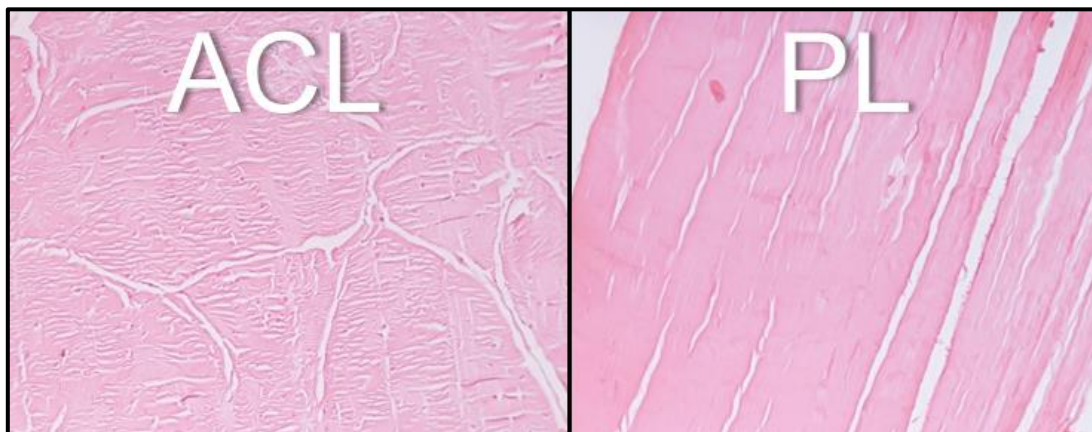


Fig. 6.1: Fibre orientation in the ACL and PL

Cell number and cell density:

The ACL and PL displayed no significant differences with regard to the number of fibroblasts present within the ligaments. This was also the case with fibroblast density. It could therefore be concluded that the ACL and PL were similar in terms of the number of cells present in a specific area. No previous literature was found to describe the number of fibroblast present in these ligaments.

Fibroblast nuclear morphology:

The ACL was composed of fibroblasts that lay in between the collagen fibres. Strocchi *et al.*³⁵ observed these fibroblasts to have elongated cell bodies. Petersen and Tillmann²⁵ found the cells in the transition zone to be oval or elongated in shape. This study observed the central region of the ACL and PL. It was found that the abundant nuclear morphology type found within the ACL was ovoid (54%) while the PL mostly consisted of spheroidal shaped nuclei (41%). Tetsunaga *et al.*⁴⁰ observed cell morphology using H&E stained sections and found fusiform fibroblast nuclei in abundance in the central region of the ACL. This is in contrast with the findings of this study, as fusiform nuclei were the least observed nuclear shape in the central region of the ACL (10%). Even though the fibroblasts found within the ACL and PL differed, it is unknown what the influence of fibroblast nuclear morphology is on the function of the ligaments.

Blood vessel presence and diameter:

Blood vessels were noted in 82% of the ACL slides analysed, while only 54% of the PL slides contained vessels. This is only valid for the small area analysed on each slide. The diameter of the blood vessels varied due to the type of vessel (arterioles, pre-capillaries, capillaries or venules) observed in both the ACL and PL. This was confirmed by Scapinelli²⁴. In almost all the cases the vessels appeared in bundles within the loose connective tissue which separates the collagen fibres. Petersen and Tillmann²⁵ also reported the blood vessels to be located in the loose connective tissue that separates the collagen fibres into bundles. The healing abilities of a ligament depend on its blood supply. No previous studies were found to confirm the microscopic blood supply of the PL.

Overall comparison of ACL and PL:

The ACL and PL were not identical in their histological composition, which could be expected, as they have different native functions, but they did show similarities. Cell number, cell density, blood supply and the fibre orientation in some areas were similar. The morphology of their fibroblasts varied, however, both contained a variation of all three types. Unfortunately, it is unknown what the influence of these

differences is, but it is still acceptable for the PL to be used as a replacement graft for an injured ACL.

6.5 Clinical relevance of this study

The length and width of the native ACL play an important role in selecting the type of graft for ACL reconstruction. Native ACL length would determine the length of graft available in the tibial and femoral tunnels for tunnel fixation. Utilising imaging modalities could allow clinicians a greater degree of accuracy in estimating ACL length from measuring the FECW of the patient. This is especially the case where a radiograph or coronal MRI is available. This would prevent the necessity of employing subsequent specialised imaging of the knee in order to visualise the entire length of the ACL. Predicting the length of the ACL pre-operatively would avoid a mismatched graft that is either too short or too long between the tibial and femoral bone plugs. This study provides a method by which patellar BTB grafts of the correct size could be ensured by determining the length of the original ACL which has to be replaced. The prevention of graft mismatch would avoid some post-operative complications such as improper graft fixation.

Future studies could address surgical techniques in which the posterolateral bundle of the ACL is re-established as current techniques do not completely replicate the ACLs anatomy and function. Most surgical techniques only address the anteromedial bundle of the ACL. Anteroposterior stability is mostly corrected, but rotational stability is often neglected. In order to restore the complete function of the ACL, the posterolateral bundle would also need to be reconstructed.

6.6 Conclusion

This study aimed to examine the macroscopic and microscopic anatomy of the ACL. The possibility to determine pre-operative ACL length, and which imaging modality is the most effective, was also studied. The anatomy of the ACL and PL of this South African cadaver sample compared favourably with results reported in previous studies conducted on other population groups. Similar to some of these studies, a correlation was found between the intra-articular ACL length and the height of the sample, but the maximum FECW of a patient was found to be the more reliable predictor of ACL length. This study also revealed that, statistically, the maximum FECW measured from either an MRI or radiograph could be used to estimate the length of the ACL. This would allow for pre-operative determination of the ideal graft length required for successful ACL reconstruction, thereby avoiding a possible graft mismatch and post-operative complications.

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