

# **A morphological and histological study of the interface between bone and the attachments of quadriceps tendon and patellar ligament**

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## **Abstract**

Knee ligaments anatomy has been recently the focus of research because of the frequent need for reconstructive arthroplastic surgery in this area. At present, the implantation of patellar tendon is regarded as a golden standard for anterior cruciate ligament reconstruction surgery. The present study was undertaken to investigate the anterior cruciate ligament and patellar tendon morphologically, histologically and radiologically to demonstrate the use of patellar tendon in autograft reconstructive knee arthroplasty. Morphology at ligament and tendon insertions were studied to establish a background for subsequent studies of insertion altered by disease and to gain impression of function based on structural relations. Morphology of quadriceps tendon insertion with special emphasis on the shape of soft tissue/bone interface and thickness of calcified fibrocartilage were studied. Specimens were taken from 50 rabbit's patellae and 40 cadaveric patellae. Using MRI taken from 50 living human subjects, the actual length relationship of knee ligaments were measured. The morphology of the interface between patellar tendon and bone was described in relation to the mechanical properties and the response of bone to stress. A description of the transformation zones that occurs in the structure of patellar tendon and ligament as they insert into the bone was demonstrated. The differences between the quantities and distribution of uncalcified fibrocartilage at the attachment of quadriceps and the origin and insertion of patellar ligament were reported. It was concluded that the implantation of the central third patellar tendon has the advantage of high primary strength when used as autograft in reconstruction of anterior cruciate ligament as it leads to minimal morbidity and high functional knee stability.

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## **Introduction**

Knee ligament anatomy and physiology have for long been the focus of research because of the frequent need for reconstructive surgery in this area. Restoration of a normal ligament bone junction (enthesis) after reconstruction is regarded as prerequisite for satisfactory results (Rodeo et al. 1993). Despite the importance of tendons in transmitting muscle force to rigid bone levers and of ligaments in maintaining structural and functional continuity of the skeleton, only few investigators have studied ligament and tendon insertions (Allegra et al. 1993).

Recent epidemiological studies of injuries indicate that the knee is the structure of greatest risk. Of the four primary ligamentous structures in the knee, the two that are injured most commonly are the anterior cruciate ligament and medial collateral ligaments especially among athletics (Hull 1997, Rovere & Adair 1983 and Golleham et al. 1985). Anterior cruciate ligament (ACL) reconstruction using the central patellar tendon autograft has been widely accepted and maybe considered the standard ideal for ACL substitute for restoring functional knee stability (Harris et al. 1997, Clancy 1985 and Franke 1985).

Ligaments are dense connective tissue structures which attach bones across joints . Following injury, ligaments do not heal by regeneration but by the formation of scar tissue similar to healing in other soft connective tissues (Frank et al.1983 and Loitz & Frank 1993).

Both normal and healing ligaments are composed of two major components, the extra-cellular matrix (the majority of which, excluding water, is composed of type1 fibrillar collagen) and ligament cells (Amiel et al. 1984 and Amiel et al. 1990). While considerable effort has gone to characterize the extra-cellular matrix in both normal and injured ligaments, information concerning the cells in these ligaments is fragmentary and as a consequence there is only a generalized understanding of them (Ian et al.2002).

Reconstruction of the anterior cruciate ligament in reconstructive knee surgery remains a source of controversy (Gao & Messner 1996). Previous reports have described the use of autogenous materials like fascia lata but today the implantation of patellar tendon with bone blocks at both ends is regarded as the golden standard for anterior cruciate ligament surgery and reconstruction providing it also with good vascularization (Clark & Stechschutte 1998, Bray et al 1988 and Dandy 1987). It has the advantage of high primary strength and rapid osseous ingrowth of the bone blocks (Butler et al. 1979).

## **Aim of the work**

The present investigation was undertaken to study the morphological features of the bone/soft tissue interface at the site of quadriceps tendon insertion and patellar ligament attachment.

This was carried out by morphological, histologic and magnetic resonance imaging studies. This work aims to evaluate the use of patellar tendon as an autograft in reconstructive knee arthroplasty.

## **Material and methods**

The human cadaveric knees used were free from any pathological conditions such as ruptures of quadriceps tendon, wrinkled appearance of the patellar ligament, patellar subluxation, malalignment or dislocation. Age of the subjects varied between 45 and 60 years.

The anatomical features of quadriceps tendon and anterior cruciate ligament were studied. Forty patellae (24 males and 16 females) with quadriceps tendon and ligamentum patellae were removed from each knee, and prepared for sectioning and examination by light microscope. Strips approximately 5 mm thick, were cut from the central portion of the attachment zone of each of the three components of quadriceps complex namely [the insertion of the quadriceps tendon into the upper pole of patella, the proximal patellar attachment of patellar ligament and the distal tibial attachment of patellar ligament] (Fig.1).

Morphometric measurements from the cadavers included the length of the most anterior part of quadriceps tendon to determine the superficial length of quadriceps tendon from the patellar base to the myotendinous junction of the rectus femoris using a millimetric scale, the length of patellar ligament and anterior cruciate ligament. Width and thickness of quadriceps tendon, patellar ligament, and anterior cruciate were also recorded. (Fig. 2).

Sections were cut at 8 mm along the long axis of the tendon or ligament and at right angles to the bone surface. For quantitative purposes, attachment zone were divided into three equal region, a. (deepest), b. (intermediate), c. (superficial) (Fig.1). The specimens prepared by light microscopy were treated for one week with 10% neutral buffered formal saline, decalcified in 2% nitric acid, dehydrated with graded alcohol, cleared in xylol and embedded in paraffin wax. Sections were stained with haematoxylin and eosin, Masson's trichrome and Verhoff van Gieson ( VVG ). The maximum height of uncalcified fibro-cartilage in region a, b and c of each specimen were measured with micrometer eyepiece at (X100). The distance from the tidemark to the furthest recognizable chondrocytes was used as a suitable comparative measure of the height of the zone of uncalcified fibro-cartilage (Evans et al. 1990).

For the electron microscope study 50 patellar tendon insertion into patellae and anterior cruciate ligament of 25 adult rabbits weighing between 3-4 kgms were used. The structural features of human and adult rabbit patellae are similar (Clark and Stehschatte, 1998). Therefore rabbit was used to obtain fresh patellae for EM studies. After anesthetizing the rabbits, the specimens were obtained, decalcified in EDTA, fixed with osmium tetra-oxide and embedded in Epon using electron microscopic techniques for fully mineralized tissue. Sections were stained with lead citrate and uranyl acetate and examined with transmission electron microscope (Joel 100 CX).

Magnetic resonance arthrograms (MR) from 50 knees (26 males and 24 females between 20 and 45 years of age) with normal quadriceps, patellar ligament and anterior cruciate ligament were done. Patients with ruptures of quadriceps tendon , anterior knee pain or chondromalacia of the patellae, knees with wrinkled appearance of the patellar ligament on magnetic resonance images, knees with patellar subluxation, malalignment or patellar dislocation were excluded.

The MR imaging was carried on patients in supine position, with the knee extended and the leg externally rotated 10 to 15° (Staeubli et al. 1999).

Sagittal imaging sequence were taken to measure the length of anterior cruciate ligament, patellar ligament, and the distance between tibial tuberosity and femoral origin of anterior cruciate ligament (ACL) .

## **Results**

Anatomical dissection of quadriceps tendon, patellar ligament and anterior cruciate ligaments of dissecting room cadavers were done (Fig. 3). To reach anterior cruciate ligament and incision was done at the medial edge of the quadriceps tendon, 3 to 4 inches above the knee, extends downward and curves gracefully around the medial edge of the patellae to end just below the tibial tubercle. The subcutaneous tissue and fascia were divided and the incision was deepened between the vastus medialis and quadriceps tendon. The capsule and synovial membrane were incised along the medial edge of this tendon, then alongside the patellae and patellar ligament. The patellae was retracted laterally and the anterior cruciate ligament was viewed clearly (McVay, 1984). The tibial attachment of anterior cruciate ligament was seen to be from a fossa in front and lateral to the anterior tibial spine. The femoral attachment of ACL was seen to be from a fossa on the posterior aspect of the medial surface of lateral femoral condyle near the articular surface. The femoral bony attachment was 16 to 24 mm in diameter. The width of ACL at the tibial end was  $11 \pm 0.2$  mm and at the femoral end was  $20 \pm 2$  mm (TABLE 1) . The anterior cruciate ligament courses anteriorly, medially and distally across the joint as it passes from the femur to the tibia. It is in addition of being intra-articular, it is also extra-synovial. Its mean length is  $3.5 \pm 1$ cm (Fig.4).

The major blood supply to ACL arises from ligamentous branches of middle genicular artery and from some terminal branches of medial and lateral inferior genicular artery (Fig.5). The ACL is covered by a synovial membrane which forms an envelope around the ligament. It is rich in vessels that originate predominantly from ligamentous branches of middle genicular artery and to a lesser extent from medial and lateral inferior genicular arteries. The synovial vessels arborize to form a web-like network of periligamentous vessels that ensheath the entire ACL. This periligamentous vessels give rise to smaller connecting branches which penetrate ACL transversely. The histological study of quadriceps tendon attached to the upper border of the patella showed that the insertion complex is formed of 4 zones: tendon, fibro-cartilage, calcified fibro-cartilage and bone (Fig.6A).

Zone 1: Is normal tendon composed of parallel bundles of collagen. Fibrocytes are located between the collagen fibers.

Zone 2 : It is the uncalcified fibro-cartilage. It is composed of parallel bundles of collagen that are continuous with those in the tendon. Chondrocytes inside lacunae surrounded by thin capsular matrix are located single or in small rows lying between the collagen. Few small blood vessel are observed in both tendon and fibro-cartilage. The junction between the uncalcified and calcified fibro-cartilage is represented by a prominent deeply basophilic line, the tidemark (Fig.6B,6C,6D). On occasions, the line maybe irregular, but it generally provides a smoother contour than that at the osteochondral junction. Chondrocytes seem to be numerous in the uncalcified fibro-cartilage and are sometimes arranged in short rows. Bundles of collagen fibers are recognizable up to osteochondral junction.

### Zone 3 ( Calcified fibro-cartilage ) and Zone 4( Bone ) :

At the origin of the patellar ligament, there was a small amount of fibrocartilage fairly uniformly distributed throughout the deep and intermediate portions of the attachment site (Fig.7A,7B,7C,7D). Collagen fibers that met the tidemark at right angles were most clearly seen here (Fig.6D). Differences in the amount of distribution of calcified fibro-cartilage broadly paralleled those described above. The calcified fibro-cartilage bone interface was most irregular at the origin of patellar ligament with interdigitation between them (Fig.7B,7C). By light microscopy, the patellar ligament usually appears to terminate at the bone at a distinct border called the cement line.

There were striking differences between the quantities and distribution of uncalcified fibro-cartilage at the attachment of the quadriceps tendon and origin and insertion of patellar ligament. By far, the largest amount of fibrocartilage was in the quadriceps tendon. Here the tissue was most characteristic of the superficial third of the attachment zone.

Light photomicrograph of rabbit's quadriceps tendon insertion into the patellae, ligamentum patellae attachment into patellae and tibial attachment of anterior cruciate ligament were demonstrated (fig.8,9,10)

As regards the electron microscopic study of quadriceps tendon insertion, ligamentum patellae and anterior cruciate ligament attachment, division of their attachment into four zones provides a convenient model for study. Although each zone possesses clearly defined characteristics, in reality the zones merge one into the other by a gradual change in morphology. Photomicrographs shows the following zones :

### **Zone 1:**

#### **Tendon or ligaments :**

These consist of more or less parallel collagen fibrils with interspersed cells. Collagen fibrils constitute by far the greatest portion of tendon and ligament. An almost parallel array of fibrils in patellar tendon and ACL produces an ideal site for ultrastructural studies. Collagen fibrils range from 25 to 140 nm in diameter. Longitudinal sections of individual fibrils expose the regular, repeating periodicity character. The period averages about 64 nm in tendon fibrils (Fig.11A). The A complex, B1, B2, C1, C2, D, E1 and E2 intraperiod bands were seen. In uranyl acetate stained fibrils, the dark portion consists of E1, E2, A complex, B1 and B2 intraperiod bands and the light portion consists of C1, C2 and D bands (Fig. 11B). A second population of fibers exhibits ultrastructural features associated with elastic fibers. They lie scattered sparsely among collagen fibrils (Fig. 12A).

#### **Ground substance :**

The extra-cellular space between collagen fibrils of tendons and ligaments presumably contains protein polysaccharides and extra- cellular fluid.

#### **Cells :**

Longitudinal elongated fibroblasts whose nucleus lies in the widest portion of the cell were present between fibril bundles. Fibrocytes were also seen.

#### **Vessels :**

A network of capillaries was seen running in a longitudinal direction between collagenous bundles. The capillary wall is composed of lining endothelium resting on a basal lamina and subendothelial connective tissue.

## **Zone 2 : Fibrocartilage**

### **Fibrils :**

Collagen fibrils and elastic fibres extend without substantial change in arrangement from tendon to form the fibro-cartilage's intercellular matrix. The collagen bundles range from 150 to 400 micrometers wide (Fig.12B).

### **Cells :**

The cells from tendon to fibro-cartilage, gradually change structural characteristics to those of the chondroblast. Its shape is oval and most cells arranged themselves in pairs or rows. Lysosomes increase. Membrane bound vesicles near the cell surface encase granular and filamentous precipitates similar to that in the extracellular matrix. Short cell processes seem to form when vesicles open onto the cell surface. The formed chondrocytes lie in lacunae. Adjacent lacunae are separated by thin bars that contain collagen fibrils. Some lacunae appear to be fully occupied by chondrocytes while others show enlarged spaces as a result of shrinkage artifacts of the chondrocytes (Fig.12C).

## **Zone 3 : Calcified fibrocartilage**

The zone, 100 to 300 microns wide is separated sharply from the previous one by the tidemark transversing the tendon almost perpendicularly to its fibers. The collagen bundles continue into this region. Mineralized crystals are situated between parallel collagen fibrils. Mineral sometimes infiltrate collagen fibrils in a manner that creates periodic dense bands which persist in the fully mineralized matrix. This results from the polymerization of tropocollagen molecules and overlapping of neighboring molecules by approximately one quarter of its length. In contrast to hyaline cartilage, fibrocartilage contains more densely packed collagen fibrils and less ground substance (Fig. 13A,13B, 14,15A).

### **Cells :**

Many chondrocytes are surrounded by calcified matrix. Some cells contain vacuolated cytoplasm, others consist of degenerated nuclear and cytoplasm fragments (Fig. 15B,15C, 15D,16).

## **Zone 4 : Bone**

Collagen fibrils in bone matrix blend with those of mineralized fibrocartilage in zone 3. No evidence of a layer separating the two. Cellular and matrix characteristics of bone beneath the tendon is similar to normal bone elsewhere (Fig. 17).

### **Cells :**

Osteoblast has a uniform appearance. The cells do not make continuous contact with each other although this may be an artifact caused by shrinkage during preparation of the material. The two components of endoplasmic reticulum can be seen clearly. There are groups of laminated membranes and small vesicles representing the Golgi complex, but this is not as well developed as that in fibroblast or chondroblast. The nuclei as well as the cytoplasm reflect the high metabolic activity of the osteoblast. Most of them have prominent nucleoli and heavy concentration of granules applied to the inner surfaces of the nuclear membrane.

Osteocytes. These mature osteoblasts have elongated nuclei and the cytoplasm contains a few organelles. The cells develop many cytoplasmic processes that

extend into canaliculi, making contact with similar process from adjacent cells.

From 40 knee cadaveric specimens using morphometric measurements demonstrated in figure 2, the length of common quadriceps tendon (CTL), width of quadriceps tendon (QW), thickness of common quadriceps tendon (CTT), patellar ligament length (PL), patellar ligament thickness (PTT) and patellar ligament width (PW) and anterior cruciate ligament (ACL), width and thickness were calculated (Table I).

Measurements were carried out on the living using MRI for comparison with those obtained from the cadavers. Distance measured from tibial tuberosity because the patellar ligament is left intact at its tibial attachment during the autograft operation (Table II).

**Table I**  
**Morphometric analysis in mm of knee ligament on human cadavers**

<b>Group</b>	<b>Mean CTL</b>	<b>QW</b>	<b>CTT</b>	<b>PL</b>	<b>PTT</b>	<b>PW</b>	<b>ACL Length</b>	<b>ACL width at femur</b>	<b>ACL width at tibia</b>	<b>ACL thickness</b>
<b>Preserved Specimen (N=40)</b>	<b>59.5 (±6.4)</b>	<b>33 (±4.5)</b>	<b>6.8 (±0.7)</b>	<b>48 (±2.5)</b>	<b>3.8 (±0.4)</b>	<b>28 (±2.9)</b>	<b>35 (±1.0)</b>	<b>20 (±2.0)</b>	<b>11 (±0.2)</b>	<b>3.0 (±0.4)</b>

CTL: Length of common quadriceps tendon.  
 QW: Width of quadriceps tendon.  
 CTT: Thickness of common quadriceps tendon.  
 PL: Length of Patellar ligament  
 PTT: Thickness of Patellar ligament  
 PW: Width of Patellar ligament  
 ACL: Anterior cruciate ligament.

**Table II**  
**Length measurements by magnetic resonance imaging (MRI) in mm on the living**

	<b>Range</b>	<b>Mean</b>	<b>SD</b>
<b>1- ACL</b>	<b>31.3 – 43</b>	<b>37.7</b>	<b>3.20</b>
<b>2- PL</b>	<b>43 – 60</b>	<b>51.50</b>	<b>5.17</b>
<b>3-Distance between tibial tuberosity and femoral origin of ACL</b>	<b>64 – 80</b>	<b>70.81</b>	<b>4.66</b>

ACL: Anterior cruciate ligament.  
 PL: Length of Patellar ligament



## **Discussion:**

Differences in the quantities of uncalcified fibro-cartilage at the insertion of quadriceps tendon and at the origin and insertion of patellar ligament found in this study were related to change in angle between the long axis of tendon or ligament and long axis of the bone during joint movement similar to what was found by (Benjamin et al 1986, Woo et al. 1988 and Evans et al. 1990). There are striking mechanical differences between quadriceps tendon and patellar ligament as the maximum force developed in tendon exceeds that in ligament by a ratio of about 8:5 because of reaction of patella against femur. Thus, the insertion of tendon is subject to a greater maximum force than in either the origin or insertion of the patellar ligament (Ellis et al. 1980, Huberti et al. 1984, Eijden et al. 1986 and Eijden et al. 1987). Evans et al. (1991) stated that the total thickness of cortical calcified tissue (thickness of both lamellar bone and calcified fibro-cartilage) was significantly greater at the insertion of quadriceps tendon than at either attachment of patellar ligament. There were also little differences in the amount of calcified tissue between upper and lower attachment of patellar ligament as was observed in this study. In the present investigation it was found that at both the origin of patellar ligament and insertion of quadriceps tendon, there was significantly less total calcified tissue in the deepest part of the attachment than in the most superficial part as was observed by Hull (1997). In the present study it was found that the total amount of bone and calcified cartilage was greatest at the superficial part of the attachment zone, where the collagen fibres had the greatest distance to travel before reaching the bone. It is also because more force is transmitted through parts of an attachment zone (superficial fibres) than others as was found by Evans et al (1991). Thus, the larger force is resisted by a greater density of bone per unit area in the quadriceps insertion as well as by an increase in the total area of attachment zone that follows from the greater thickness of the tendon. It is suggested that the larger amount of fibro-cartilage in the quadriceps tendon may be related to the greater absolute size of the tendon compared with the ligament. The close similarity in the amount of bone and calcified fibro-cartilage at the origin and insertion of the patellar ligament and both ends of ACL found in this study can be explained by the identical force at either end of this ligament and was similar to observation of Evans et al (1991). Regarding the patellar ligament attachments, Pedley & Meachim (1979) found a greater density of bone and total calcified tissue on the lateral compared to the medial side of patella. It may be due to mechanical differences at the two regions. The significance of the fibro-cartilage is possibly that it has a mechanical role diffusing forces over the entire attachment site. This minimizes local concentration of stress. The fibro-cartilaginous zones may prevent fatigue failure by providing a more gradual transition from soft tissue to the hard bone. It is suggested that the shape of the soft tissue-bone interface and the thickness of calcified fibro-cartilage may be capable of withstanding tensile loads.

The fact that no mechanical failure occurred at the irregular tidemark line, but rather through the zone of calcified cartilage or sub-chondral bone may underline the effectiveness of inter-digitations between 2 different types of tissue to resist mechanical forces (Crowninshield & Pope 1976).

The uncalcified fibro-cartilage ensures that the tendon fibres do not blend, splay out or become compressed at a hard tissue interface and offer protection from wear and tear (Benjamin et al. 1986). In this study, it was seen that tendon fibres inter-digitated among bone lamellar systems but did not merge with the collagen system of individual lamellae like what observed by Clark et al. (1998) .

Structural alternations at insertions were reported during normal development. Increasing age weakens the tissue-bone unit. The frequency of ACL injury in athletes has led to the increasing use of biologic grafts. Selection of a biologic substitute requires detailed knowledge of its anatomical and histological structure (Lacroix 1951).

The central quadriceps tendon is thicker and wider than patellar ligament thereby providing a plentiful source of tendon for ACL reconstruction. Harvesting this graft is demanding attention to details. This will help the surgeon to have minimal morbidity (Butler et al. 1985).

Noyes et al. (1983) found that 14 mm of patellar tendon bone has 175% of the strength of ACL. Vascular studies have demonstrated that either the inferior lateral genicular artery or inferior medial genicular artery can be used with patellar tendon to produce a vascularized graft. The patellar tendon autograft has the advantage of possessing a greater tensile strength. The removal of its 1/3 does not sacrifice its use as a stabilizer for the knee (Clancy et al. 1982, Paulos et al. 1983, Noyes et al. 1984 and Bray et al. 1988).

Orthopedic surgeons described the use of central 1/3 of patellar tendon. The graft consisted of a triangular block of bone from superficial portion of the patella, strip of quadriceps taken one inch above superior pole of patella and 1/3 patellar ligament. This graft was left attached distally and passed to the joint through a notch beneath the fat pad. The graft was pulled through a tunnel in the lateral femoral condyle and sutured to periosteum of distal femur (Jones 1963 and Jones 1970). (Fig.19)

It was concluded that this investigation gives some knowledge that will allow the clinician to make the necessary intra-operative and post-operative decisions in ACL reconstruction. It will establish a background for subsequent studies of insertion altered by disease and to gain impressions of function based on structural relation.

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## Legands

**Figure 1:** The attachment zones studied are the insertion of quadriceps tendon (QT), the origin and insertion of patellar ligament (OPL), (IPL) respectively. The subdivision of each site into regions a, b, c and the major differences in the quantities and distribution of uncalcified fibro-cartilage (uf) are illustrated diagrammatically in the drawings to the left of the figure. F: Femur, T: Tibia, PL: Patellar ligament. The attachment zones were divided into three equal regions: a) Deepest b) Intermediate c) Superficial

**Figure 2:** **A:** Frontal view of the knee region with morphometric parameters shown. **B:** Lateral view of the knee region with morphometric parameters shown. QW: Width of quadriceps tendon, PTW: Patellar ligament width, CTL: length of common quadriceps tendon, CTT: Thickness of common quadriceps tendon, PTL: Patellar ligament length and PTT: Patellar ligament thickness.

**Figure 3:** Photographs of human cadaver's knee. **A:** Shows the femur (F), the anterior cruciate ligament (ACL), patellar ligament (PL) and patella (Pa). **B:** Shows the quadriceps (Q) with its myotendenious junction (MJT) which appears oblique and represented by red pins and the patella (Pa). (Qt) is the quadriceps tendon. The black pins represent the upper end of patella. **C:** Midsagittal section of the knee region, the quadriceps tendon (Qt) and the patellar ligament (PL). The red pin points to the supra-patellar bursa (SB), the (if) is the infra-patellar pad of fat and (Pa) is the patellar bone.

**Figure 4:** Diagram of the ACL in extension and flexion. In extension, the posterolateral bulk is taut, while in flexion, the anteromedial band is taut and the

posterolateral bulk is relatively relaxed. AA': anteromedial band and BB': posterolateral bulk.

**Figure 5:** Photographs demonstrating the major blood supply to the human cadaveric anterior cruciate ligament (ACL) arising from the ligamentous branches of the middle genicular artery (MGa) and from terminal branches of the medial inferior genicular artery (MIGa). (Pa) is popliteal artery. **A:** Unstained vessels. **B:** Stained vessels. **C:** Diagram showing the blood supply.

**Figure 6:** Light photomicrographs of a section of common quadriceps tendon insertion into the human cadaver's patellar bone (b). **A:** Shows the four zones: (1) collagen fibres (Co) of quadriceps tendon, (2) zone of uncalcified fibro-cartilage (uf) (3) zone of calcified fibro-cartilage (cf) which stains darker than the previous zone, and (4) the bone (b). H&E (X40). **B:** Magnification of the previous section showing the patellar bone (b) stained reddish and containing almond-shaped osteocytes. The calcified fibro-cartilage (cf) and uncalcified fibro-cartilage (uf) meet at the tidemark (T) where the chondrocytes (ch) are more numerous in the uncalcified fibro-cartilage than the calcified fibro-cartilage. H&E (X400). **C:** Shows rows of chondrocytes (ch) more apparent in the uncalcified fibro-cartilage (uf). Muscle fibres of quadriceps (mf) changes to collagen fibres (Co) as they approach the tidemark (T). Osteocytes (o). Masson trichrome (X400). **D:** Shows variation in the staining property of the calcified fibro-cartilage (cf) (light orange) from that of the uncalcified fibro-cartilage (uf). (Co): Collagen fibres of quadriceps tendon. (T): Tidemark. VVG (X32).

**Figure 7:** Light photomicrographs of a section of the upper end of human patellar ligament. **A:** Demonstrates the collagen fibres (Co) of patellar ligament, the uncalcified fibro-cartilage (uf), tidemark (T), the calcified fibro-cartilage (cf) and patellar bone (b). H&E (X100). **B:** The four zones are seen: The collagen fibres (Co) of the patellar ligament (Zone 1), the uncalcified fibro-cartilage (uf) with its chondrocytes (ch) (Zone 2), the calcified fibro-cartilage (cf) (Zone 3). An irregular tidemark is seen between the 2<sup>nd</sup> and 3<sup>rd</sup> zone as a result of interdigitation between calcified and uncalcified fibro-cartilage. The patellar bone (b) containing osteocytes (o) (Zone 4). H&E (X400). **C:** Demonstrates the site of attachment of patellar ligament into the patellar bone (b). Note the extensive interdigitation between uncalcified (uf) and calcified fibro-cartilage (cf) and between calcified fibro-cartilage (cf) and bone (b). Masson trichrome (X32). **D:** Shows the extensive interdigitations (Id) between the blue calcified fibro-cartilage and the red bone (b) at the bone-soft tissue interface (Co): Collagen fibres. (ch): chondrocytes inside lacunae. Masson trichrome (X100).

**Figure 8:** Light photomicrograph of quadriceps tendon insertion into the patella (b) of a rabbit. (cf) is the zone of calcified fibro-cartilage and (uf) is the zone of uncalcified fibro-cartilage and (T) is the tidemark. (Co) is the collagen fibres of (ACL). (mf) muscle fibres of quadriceps. The tidemark is regular and smooth. The mineralization is quite abundant in the calcified fibro-cartilage. Masson trichrome (X100).

**Figure 9:** Light photomicrograph of the upper attachment of adult rabbit's ligamentum patella into the patella (b). The collagen fibres (Co) run a parallel course. The broken lines indicate the extent of the uncalcified (uf) and calcified (cf) fibro-

cartilage. The calcified fibro-cartilage is continuous with the subchondral bone (b). H&E (X100).

**Figure 10:** Light photomicrograph of tibial attachment of (ACL) of adult rabbit.

**A:** The collagen bundles (Co) of the ligament interdigitate with the uncalcified fibro-cartilage (uf). The tidemark (T) between the uncalcified fibro-cartilage and calcified fibro-cartilage is smooth and regular. The boundary between the calcified fibro-cartilage (cf) and bone (b) is highly irregular. Blood vessels in volkman's canals (v) are apparent. Masson trichrome (X100). **B:** The four zones characteristics of such an insertion site are present. The collagen fibres (Co) of the ACL are continuous with those of the uncalcified fibro-cartilage (uf). The calcified fibro-cartilage (cf) is identified by a darker matrix and contained chondrocytes (arrow). The osteons (o), (the Haversian systems) of the tibia are apparent. (Co) of ACL approach but never penetrate the tibia (b). Toluidine blue (X100).

**Figure 11:** Transmission electron photomicrograph of zone 1 (quadriceps tendon) of adult's rabbit. **A:** Shows fibroblast in between parallel longitudinal bundles of collagen fibrils (Co). Observe the fine striations of collagen fibrils. (N): nucleus of fibroblast, (m) mitochondria in the upper left side of nucleus and (cp) cell processes in contact with extra-cellular matrix. Magnification (X18500). **B:** Note the cross banding of individual fibrils. Magnification (X32000).

**Figure 12:** Electron photomicrograph of a cross section of ACL of the rabbit. **A:** Shows elastic fibres (E) at two locations. Collage fibrils (Co) comprise the remainder of the tendon. Magnification (X10700). **B:** Photomicrograph of un-mineralized fibro-cartilage of ACL showing chondrocytes in lacuna (La) and nucleus (N). Lysosomes are seen in the cytoplasm (arrow). Collagen fibrils (Co) are seen running in various directions. Magnification (X4000). **C:** A section of quadriceps tendon showing a chondroblast in the process of matrix synthesis. The cell is rounded with numerous cytoplasmic processes (cp). The nucleus (N) is generally rounded with few indentations and containing condensed chromatin. The cell is embedded in bundles of collagen fibres (Co) that run longitudinally. Magnification (X8000).

**Figure 13:** Electron photomicrograph of zone 3 of a rabbit's quadriceps tendon (the mineralized fibro-cartilage). **A:** shows the typical appearance of type I collagen. Its characteristic feature is a pattern of cross banding of fibrils with a periodicity of 64 nm. In the inset, the A complex, B1, B2, C1, C2, D, E1 and E2 intra-period bands stand out clearly. E1, E2, A, B1, and B2 bands comprise the dark portion of the 64 nm period and C1 and C2 and D bands comprise the light portion. Mineral (arrow) of mineralized fibro-cartilage is seen between or on the surface of collagen fibrils. Magnification (X32000). **B:** Photomicrograph of a capsular matrix of quadriceps tendon showing collagen fibres (Co), and a network of elastic fibres (E) with granular mineral clouds (M) in zone 3 (mineralized fibro-cartilage) in the interstices. Magnification (X25000).

**Figure 14:** Electron photomicrograph of a rabbit's patellar ligament showing the zone between mineralized calcified fibro-cartilage (cf) (dark) and unmineralized fibro-cartilage (uf) (light). (Co) is collagen fibres. Magnification (X2600).

**Figure 15:** Electron photomicrograph of adult rabbit's quadriceps tendon insertion. **A:** Shows the junction of unmineralized (uf) and mineralized calcified fibro-cartilage (cf)

and the tidemark (T) of quadriceps tendon. In the first mineralized fibro-cartilage region, mineral (arrows) lies between parallel collagen fibrils. In the deeper region, mineral (M) lies both in and between fibrils. Magnification (X16500). **B:** Early mineralized fibro-cartilage of a section zone of quadriceps tendon (zone 3) demonstrating a part of chondrocyte, nucleus (N) with hetero-chromatin in the periphery, cytoplasmic processes (cp), rough endoplasmic reticulum (rER). Masses of electron dense deposits are seen within the matrix and collagen fibrils (Co). The lacuna of chondrocyte is bounded by fine collagen fibrils (F) in the capsular matrix. Short branching processes extend from the cell where vesicles open onto the surface. (NP) is the nuclear pore. Magnification (X32000).

**C:** Intermediate stage of mineralized fibro-cartilage of a section of quadriceps tendon (zone 3). Electron dense collagen fibrils (Co) are evident in the capsular matrix of the chondrocyte. The inter-capsular matrix (IM) is devoid of electron small dense material. An electron opaque line (Ol) delineate the periphery of capsular matrix. Note the nucleus of chondrocyte (N), mitochondria (m) and the vesicles (v). Magnification (20000). **D:** Late mineralized fibro-cartilage of a section of quadriceps tendon (zone 3). In comparison to the previous view, this photomicrograph shows prominent electron dense deposits (M) surrounding the lacuna (La). Cell process (cp), filaments (F) and nucleus of chondrocyte are evident. Magnification (X16500).

**Figure 16:** Electron photomicrograph of tibial attachment of a rabbit's anterior cruciate ligament. It shows the early formation of calcified fibro-cartilage (zone3). Note that the chondrocyte is located inside the lacuna (La). The capsular (CM) and inter-capsular matrix (IM) are evident. Scattered dense deposits (M) are occasionally seen.

**Figure 17:** Electron photomicrograph of (zone 4) of quadriceps tendon insertion into a decalcified section of a rabbit's patella. It shows osteocytes with hetero-chromatic nucleus (N) and a prominent nucleolus (nu). Cytoplasmic processes (cp) are clearly evident. Lysosomes are observed in the lower left side. The osteocyte is surrounded by bone tissue (bt), which in turn is surrounded by the collagenous tissue (Co). Magnification (X14500) .

**Figure 18:** Midsagittal magnetic resonance imaging proton density of the left living human knee region showing the length of patellar ligament (PL) and anterior cruciate ligament (ACL).

**Figure 19:** [A] The central one third of the patellar ligament used to reconstruct the anterior cruciate ligament.

[B] The new ligament is pulled into the lateral femoral tunnel and placed beneath the fat pad.