Light and electron microscopic study of the medial collateral ligament epiligament tissue in human knees

Georgi P Georgiev, Alexandar Iliev, Georgi Kotov, Plamen Kinov, Svetoslav Slavchev, Boycho Landzhov

Abstract

AIM
To examine the normal morphology of the epiligament tissue of the knee medial collateral ligament (MCL) in humans.

METHODS
Several samples of the mid-substance of the MCL of the knee joint from 7 fresh human cadavers (3 females and 4 males) were taken. Examination of the epiligament tissue was conducted by light microscopy and photomicrography on semi-thin sections of formalin fixed paraffin-embedded blocks that were routinely stained with haematoxylin and eosin, Mallory stain and Van Gieson's stain. Electron microscopy of the epiligament tissue was performed on ultra-thin sections incubated in 1% osmium tetroxide and contrasted with 2.5% uranyl acetate, lead nitrate, and sodium citrate.

RESULTS
The current light microscopic study demonstrated that the epiligament of the MCL consisted of fibroblasts, fibrocytes, adipocytes, neuro-vascular bundles and numerous multidirectional collagen fibers. In contrast, the ligament body was poorly vascularised, composed
of hypo-cellular fascicles which were formed of longitudinal groups of collagen fibers. Moreover, most of the vessels of the epiligament-ligament complex were situated in the epiligament tissue. The electron microscopic study revealed fibroblasts with various shapes in the epiligament substance. All of them had the ultrastructural characteristics of active cells with large nuclei, well developed rough endoplasmic reticulum, multiple ribosomes, poorly developed Golgi apparatus, elliptical mitochondria and oval lysosomes. The electron microscopy also confirmed the presence of adipocytes, mast cells, myelinated and unmyelinated nerve fibers and chaotically oriented collagen fibers.

**CONCLUSION**

Significant differences exist between the normal structure of the ligament and the epiligament whose morphology and function is to be studied further.

**Key words:** Knee; Epiligament; Knee medial collateral ligament; Electron Microscopy; Humans; Microscopy; Photomicrography

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Core tip: The epiligament of the medial collateral ligament of the human knee is an important enveloping supporting structure of the ligament proper containing fibroblasts, fibrocytes, adipocytes, mast cells, and neurovascular bundles in a network of collagen fibres that is not limited to the surface of the ligament but also pervades it, as the endoligament, thus providing the cellular elements and blood vessels that participate in the ligament's nutrition and during the process of healing.

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**INTRODUCTION**

The medial collateral ligament (MCL) of the knee joint, also known as tibial collateral ligament (TCL), is an often injured ligamentous structure of the knee joint [1-3]. Ninety percent of knee ligament injuries involve the MCL or the anterior cruciate ligament (ACL) [4]. The incidence of this injury has increased in recent years and presents a commonly encountered problem in modern sports medicine [5,6]. Most injuries result from a valgus force on the knee from direct contact or with cutting manoeuvres, namely when athletes place a foot in a stable position and then rapidly change the direction of movement [7]. The popularity of sports such as football, skiing and ice hockey has also contributed to the increased incidence of MCL injuries [8]. According to the model of Warren and Marshall, the medial knee is divided into the following three layers: Superficial (I), intermediate (II), and deep (III) [9]. The superficial layer (I) consists of the deep crural fascia which invests the sartorius and quadriceps and continues into the deep fascia of the lower extremity, where it covers the gastrocnemius and the popliteal fossa. Layer II, or the intermediate layer, includes the superficial MCL (sMCL) and medial patellofemoral ligament (MPFL). Layer III is the deep layer and comprises the joint capsule and the deep MCL (dMCL). The superficial and dMCL have similar functions and act as the primary supporting structures of the medial side of the knee [10,11], therefore injuries to these structures merit due attention and adequate treatment [12]. The healing of ligaments after injury is associated with scar tissue formation rather than regeneration [13-15].

The structure of the MCL has been studied extensively, however, very little is known about the thin layer of connective tissue adherent to this ligament, termed the epiligament (EL) [epi-(Greek-on or upon); ligament (Latin-ligare, to bind)]. In 1990, Bray et al. [15] described the epiligament as a "surrounding adherent connective tissue removed simultaneously with the ligament but which was grossly distinguishable from ligament tissue proper". Our previous studies on the MCL in rat knee models led to the conclusion that the EL tissue plays a key role in the healing of the ligament tissue after injury [16,17]. According to Georgiev and Vidinov [18-20] and Georgiev et al. [21,22] the EL is a donor of fibroblasts, progenitor cells and blood vessels, which proliferate and migrate towards the body of the ligament through the epiligament during the process of ligament recovery. Fibroblasts in the EL tissue normally produce collagen types I, III, V, fibronectin (FN) and matrix metalloproteinases-2 and -9 (MMP-2, -9) which are essential for the normal functioning of the ligament and their synthesis is increased in order to promote adequate repair after injury [13,16,17,21,22]. Therefore, detailed knowledge of the morphology and function of the EL during physiological conditions and post injury is important in deepening our knowledge with regard to ligament healing and may thus lead to proposal of better treatment options in the future. There is plentiful literature data concerning the role of the EL during MCL healing in rats, however its normal anatomy in humans has not been studied yet. In line with this, the aim of this study is to investigate the normal morphology of the MCL EL in humans for the first time in the literature, through light and electron microscopy and to compare it to the ligament substance.

**MATERIALS AND METHODS**

Several samples of the mid-substance of the MCL of the knee joint of 7 cadavers (3 females and 4 males) were taken from the fresh human cadavers available at the Department of Anatomy, Histology and Embryology at
Normal morphology of the MCL EL: Electron microscopy

The electron microscopy revealed the presence of various types of fibroblasts in the EL: Spindle-shaped, spindleshaped, elongated and fibroblasts with irregular shape. They had large nuclei, well developed rough endoplasmic reticulum, multiple ribosomes, poorly developed Golgi complex, individual elliptical mitochondria and oval, individually located lysosomes (Figure 2A-C). The electron microscopy also manifested the presence of adipocytes and mast cells (Figure 1F). The mast cells had well-presented nuclei with peripheral heterochromatin. The cytoplasm contained the specific round or oval granules. The granules were always enclosed by a membrane and separated from other granules by cytoplasmic septa. The matrix of each granule was homogeneous and electron-dense. The electron microscopy revealed that the adipocytes had large lipid droplets which pushed the rest of cytoplasm at the periphery of the cell. The nuclei of the adipocytes were eccentrically located.

Collagen fibres in the EL of the midportion of the MCL were quite similar in diameter and were positioned in bundles with various orientation.

Discussion

The ligament is built of connective tissue, which comprises two main elements-connective tissue cells and extracellular matrix[11,24]. Collagen fibres in the ligaments are organized in longitudinal groups and form fascicles[11,24,25]. The thin layer of connective tissue separating these fascicles is known as epiligament and is related to another connective tissue structure, containing more blood vessels, which envelops the entire ligament and is known as epiligament[12,13,16-18]. In rabbits, Chowdhury et al[26] (1991) examined the external surface of the MCL EL and described two types of cells - spindleshaped adipocytes and fibroblasts. It is fibroblasts that produce collagen fibres and thus are responsible for the formation of scar tissue[28]. In rats, Georgiev et al[12,13,16,17] showed the external portion of the MCL EL to consist of fibroblasts, fibrocytes, adipocytes, neurovascular bundles, and a number of collagen fibres, oriented in varying directions. These cells are located among bundles of collagen fibres. Georgiev et al[21] also described the ultrastructural characteristics of the different types of fibroblasts in the EL of the lateral collateral ligament (LCL) in rat knees. In terms of shape, they described spindle-shaped fibroblasts, small elongated fibroblasts and fibroblasts with irregular shape. All of these cells were characterized by the presence of a large nucleus with prominent nucleoli, well-developed rough endoplasmic...
reticulum and numerous ribosomes. These ultrastructural characteristics led Georgiev et al.\cite{22} to conclude that fibroblasts in the EL might take part in the differentiation, phagocytosis and collagen synthesis, possibly thus playing a role in the regeneration of the ligament after injury, which has also been proposed by other authors\cite{11}. Moreover, fibroblasts in the EL may proliferate and migrate through the endoligament into the ligament proper\cite{18,27}.

Other rarely observed types of cells are mast cells which have an oval shape and numerous granules with homogeneous density\cite{22}. Adipocytes are organized in cellular lobuli, enveloped by thin connective tissue fibres and represent the building blocks of white adipose tissue\cite{24}. According to Chowdhury et al.\cite{28} adipocytes synthesize, process and store lipids and thus participate in nutrition and confine specific storage and protective functions to the EL.

In humans, our light microscopic and ultrastructural study confirmed the aforementioned characteristics of the EL tissue and its constituent cells. On light microscopy, we noted the existence of fibroblasts, fibrocytes, adipocytes, neuro-muscular bundles and numerous multidirectional collagen fibres. This greatly resembled the structure of the EL observed in rats\cite{22}. Also, we observed that the main cytological features of the EL were closely related to those in the synovium. This provides further support to the theory that the EL is a specialised form of synovium\cite{11,28}. Electron microscopy revealed a great variety of fibroblasts in terms of shape - spindle-shaped, spinous-shaped, elongated and irregularly-shaped, which confirmed earlier results in rats\cite{22}. We found an abundance of structures in their cytoplasm, namely a well-developed rough
endoplasmic reticulum and multiple ribosomes, which supports the hypothesis that fibroblasts play a key role in the ligament nutrition and healing after injury.\[11,22\].

As in the rat and the rabbit, the EL tissue in humans appears to contain a relative abundance of blood vessels.\[12,24,26,29,30\]. Blood vessels in the EL are randomly dispersed in an amorphous structure, built of loose connective tissue.\[26,30\]. They branch extensively, forming anastomotic networks of interconnected vessels.\[29,30\]. Blood vessels in the EL are often accompanied by nerve bundles, but apparently not all blood vessels are organized in a neurovascular bundle.\[15,26,30\].

The healing of ligaments after injury is associated with scar tissue formation rather than regeneration, which shows common mechanisms to the healing processes in other soft tissue structures.\[31-34\]. According to Frank et al.\[21\] injury location has an impact on ligament healing. The MCL heals much better and faster than the ACL of the knee joint. This is most likely due to the specific characteristics of the EL, located above the MCL. Georgiev and Vidinov,\[18-20\], Georgiev et al.\[13,16,17,21,22\] and Lo et al.\[29\] claim that the EL may be the primary donor of connective tissue cells participating in the scar formation as part of the process of ligament healing. Fibroblasts are not static cells and as such can migrate from the EL to the healing ligament.\[12,13,21,22,26\]. According to Chamberlain et al.\[27\], ligament injuries stimulate the migration of various cell types from the EL, including neutrophils and cells in the process of mitosis up to the fifth day after injury, which proves that there is a bilateral cooperation between the EL and the ligament with regard to adequate healing of the ligament.

In conclusion, this study illustrates in detail the normal morphology of the MCL EL in humans and demonstrates...

Figure 2  Normal morphology of the medial collateral ligament epiligament tissue in human. A-C: Electron micrograph of a fibroblast (Fb) and its nucleus (N). Mitochondria, lysosomes and rough endoplasmic reticulum are visible in the cytoplasm of the Fb; in the extracellular matrix numerous collagen fibers (col) are presented × 7000; D and E: Electron micrograph of EL collagen fibers (col) in the extracellular matrix oriented in different directions × 7000, × 9000; F: Electron micrograph of a fibroblast (Fb) and its nucleus (N) and a mast cell (Mc) with numerous granules and its nucleus (N), × 9000.
its difference from the structure of the ligament tissue for the first time. The electron microscopic study reveals the specific characteristics of the various types of cells in the EL and supports the hypothesis that fibroblasts in particular, together with the abundant blood vessels are essential for the nutrition and healing of the MCL.

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