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The Epiligament-The Main Donor of Cells and Vessels during Healing of the Collateral Ligaments of the Knee

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Abstract

Ligaments are composed of dense connective tissue and attach bones in joints. The thin connective tissue sheath, covering these fascicles is called endoligament and is connected to a more vascular connective tissue structure that envelopes the entire ligament and is referred to as epiligament. The tissue of the epiligaments is composed of different cell types such as: fibroblasts, fibrocytes, adipocytes, neurovascular bundles, and a multitude of collagen fibers, disposed in different directions. The main structural protein of epiligament is collagen type I. Collagens types III and V were also found in the structure of epiligament. Type I collagen is the main collagen in normal and healing ligaments. The ligament repair requires presence of collagen type III. Collagen type V is associated with collagen type I and regulates the collagen fibril diameter. Knowledge of variation of cells and collagen types of epiligament in normal and injured ligaments is crucial for understanding of the healing process.

Keywords: Collateral ligament epiligament; Knee

Abbreviations:

EL: Epiligament; MCL: Medial Collateral Ligament

Ligament and Epiligament (EL) Structure

The structures, composed of dense connective tissues that attach bones in joints are known as ligaments. The main components of ligaments connective tissue are ligament cells and extracellular matrix [1,2]. The internal gross organization of ligaments includes fascicles which are composed of collagen fibers arranged in longitudinal groups [3]. The thin connective tissue sheath, covering these fascicles is called endoligament and is connected to a more vascular connective tissue structure that envelopes the entire ligament and is referred to as epiligament (EL) [4].

The first definition of the term in the scientific literature is given by Bray et al. [5], described it as "surrounding adherent connective tissue removed simultaneously with the ligament but which was grossly distinguishable from ligament tissue proper". In the external area of medial collateral ligament (MCL) in rabbits only two types of cells were observed – spinous and cuboidal shaped fibroblasts and fat cells [4]. In addition Georgiev et al. [6,7] described different types of fibroblasts – spinous-shaped, spindle-shaped, elongated, irregular in shape and fat cells.

There are relatively few data about the structural and physiological features of the EL, therefore herein we review the existing data about this structure in the literature and lay emphasis on its clinical significance.

Macroscopic Appearance

The EL is a thin translucent layer which entirely covers the ligament and in the same time mobile in all directions. Its compliance is higher than that of the underlying ligament. At the ligament insertion sites the EL is continuous with the periosteum. It is attached to the MCL by fronds of synovium. In the deeper areas which are towards the joint cavity, the EL is attached tighter to the ligament than in the superficial ones. The EL is more difficult to distinguish from MCL in immature animals. There is a great quantity of small caliber blood vessels in the EL of the medial side of MCL. The vessels most commonly cross the epiligament in an oblique angle. An arborisation of the geniculate venous tree is observed crossing the distant third of the ligament in an oblique direction, but its position might be varying [4]. According to Bray et al. [5], ligaments of the knee show similarities in the way blood vessels and the epiligamentous plexuses arborised in the junctional region between outer surface of ligament and epiligament. Different densities of these vessels have been observed along the length of ligaments. Majority of epiligamentous vessels from ligaments are longitudinally orientated in the border of the epiligament and superficial layers of the ligament, afterward penetrate deeply the ligament, forming anastomses and situated along to the axis of ligaments.

Microscopic Appearance

Types of cells (light, electron microscopy)

The external surface of the EL of the MCL in rabbits was examined firstly by Chowdhury et al. [4], who found two types of cells – spinous and cuboidal shaped fibroblasts and adipocytes. The latter compose the ligament scar tissue and are mostly responsible for the collagen fibers synthesis. According to Georgiev et al. [9,10] the external aspect

of the EL consists of fibroblasts, fibrocytes, adipocytes, neurovascular bundles, and a multitude of collagen fibres, disposed in different directions (Figures 1 and 2). The EL of MCL ligament tissue is more vascularized and composed of ligament hypocellular collagen fascicles. The cells are interspersed between bundles of collagenous fibres. The cells of the EL have a higher density than other connective tissues. In the transition area the cells have a rounded shape. There are many single cells and groups of cells on the lateral side of the EL. Different types of fibroblasts with well-developed granular endoplasmic reticulum have been established (Figure 3). Georgiev et al. [9,10], stated that the EL is composed of different types of fibroblasts: with large nuclei and well visible nucleoli and long cytoplasmic processes; spindle shaped fibroblasts; small elongated fibroblasts, fibroblasts with irregular form. Rarely are visible mast cells with oval shape and many homogeneous dense granules. Well-developed rough endoplasmic reticulum and many ribosomes have been observed in the cytoplasm of fibroblasts. The EL adipocytes are typical fat cells which are the main elements of white adipose tissue [11]. Chowdhury et al. [4] claim that these adipocytes metabolize and store lipids and their function is to endue specific substance properties to the EL.

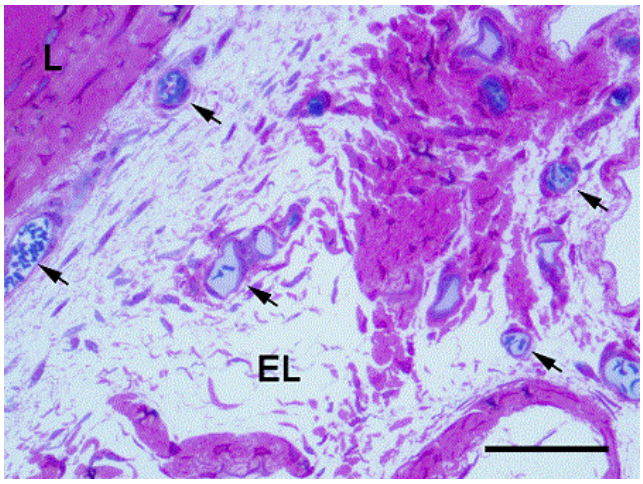

Figure 1: Epiligament (EL) – ligament (L) - normal morphology: EL consists of fibroblasts, fibrocytes, adipocytes, and a multitude of collagen fibres, disposed in different directions. H&E. Bar 100 μm.

Difference between EL and L

Both epiligament and endoligament have certain features, distinguishing them from the ligament [4]. These structures are more cellular then ligaments and are arranged mostly perpendicularly to the ligament axis. This arrangement supplies a cover mesh for each fascicle and the whole ligament, which is the structural subject of formation of a functional unit [12].

The amount of cells in EL is greater than those of the ligament. The quantity of blood vessels and nerves is also more in number [9,11,13,14]. Growing cells in vitro leads to parallel arrangement of them to the long axis of the tension, and in the same time the cells orientate away from it [13]. The described arrangement is similar to a great extent to that in the EL in vivo, in which the cells are in perpendicular position to the longitudinal axis of the ligament [12].

Blood vessels in the EL are positioned in a loose connective tissue matrix. Usually there are nerve bundles adjacent the blood vessels in the EL, but not all blood vessels are part of a neurovascular bundle [4,14].

In accordance with the studies of Bray et al. [14], myelinated and unmyelinated nerve fibers are more commonly presented in the EL tissue in comparison to the ligament. The position of the nerve fibers most commonly is parallel to the collagen strands in the EL. The nerves into the main substance of the ligament might be seen as free nerve endings or as elements of neurovascular bundles, which branch off in the deeper layers.

By light microscopic examination of EL, it is established that its general cellular morphology is akin to that of the synovium which is compatible with the theory that the EL is a specialized structure of synovium [12].

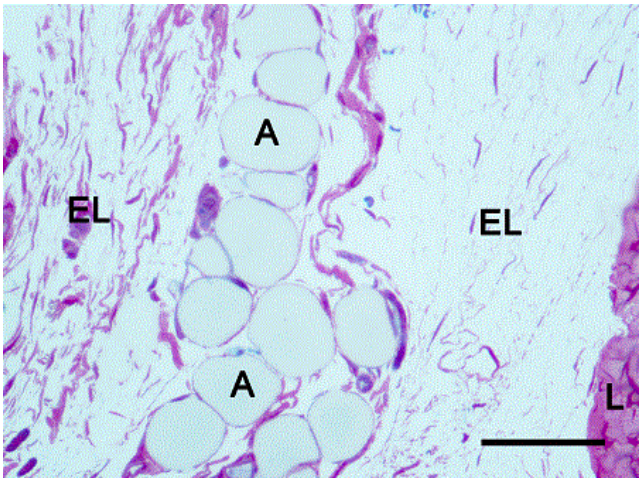

Figure 2: Epiligament (EL) - normal morphology: EL consists of fibroblasts, fibrocytes, adipocytes, and a multitude of collagen fibres, disposed in different directions. L-ligament, A-adopocyte. H&E, Bar 50 μm.

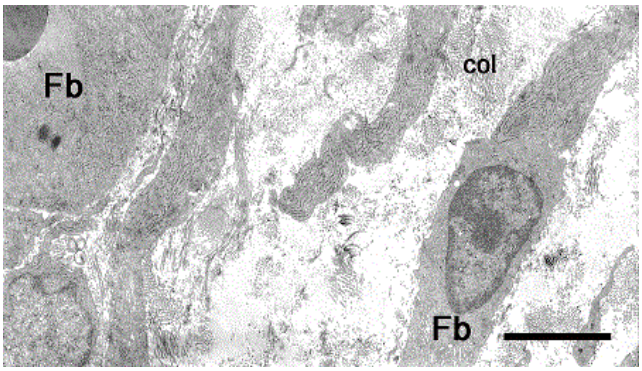

Figure 3: Transmission electron microscopy of the epiligament-normal morphology.Different types of fibroblasts (Fb) with well-developed granular endoplasmic reticulum are visible. col – collagen. Bar 2 μm.

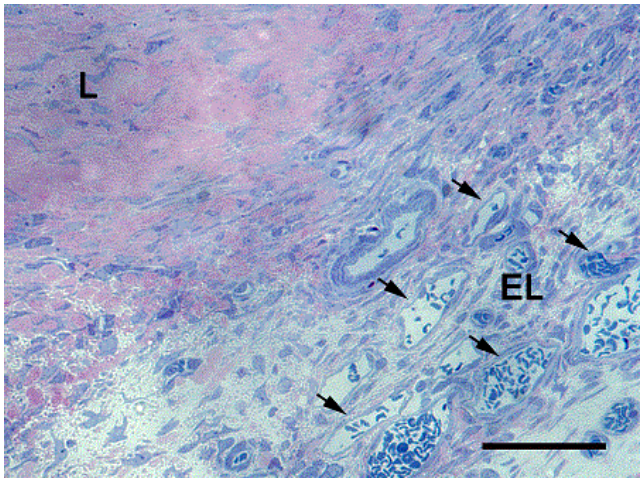

Figure 4: Epiligament (EL) – ligament (L) at the eight day after injury: The scar region was characterized as hyper-cellular and showed intensive angiogenesis. Blood vessels (arrows). H&E. Bar 100 μm.

Light microscopic examination showed that the general cellular morphology of EL was similar to that seen in synovium, which coincides with the hypothesis that the EL is a specialized form of synovium [12].

Types of Collagens in the EL

It is known that the main structural protein of EL is collagen type 1 [9,10,15]. Immunostaining showed presence of type I collagen in both epiligament and ligament tissue. Type I collagen was also detected in the adventicia of the blood vessels in the EL. In healthy EL a small amount of immunopositive reaction for type III collagen was detected in tunica media of the blood vessels. The collagen type V was localized only in the epiligament [9,10].

Presence of collagen type I and V is described in the EL. It was observed that the thick collagen fibrils split off in thinner fibers [9]. Type I collagen is the main collagen in normal and healing ligaments. It is known that it gives the ligaments tensile strength and is crucial for the long-term features of the tissue matrix [16,17]. According to Niyibizi et al. [18], the ligament repair requires presence of collagen type III. High elevation of production of collagen type III as compared to collagen type I in phases of early ligament healing is detected. Liu et al. [19] and Breuls et al. [20] state that collagen type V is associated with collagen type I and regulates the collagen fibril diameter. The enhanced levels of type V collagen influence the stiffness of the extracelular matrix by changing the fibril organization of extracellular matrix [21]. A great number of collagens as collagen type VI, IX, X, XI, XII, and XIV have also been established in the ligament tissue [16].

EL Role during Ligament Healing (Light, Electron Microscopic and Immunohistochemical Study)

Healing of ligaments after injuries is provided by formation of scar tissue, which is quite similar to the healing processes in other soft tissue structures [22-25]. In process of healing of ligaments, MCL scars showed increased number of cells [2,12,23]. According to Frank et al. [23] injury location affects ligament healing.

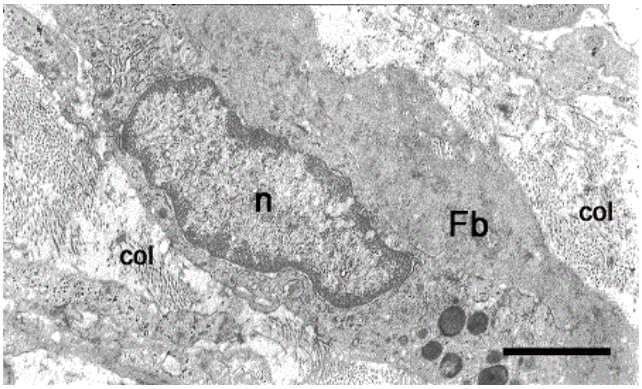

Figure 5: Transmission electron microscopy of the epiligament at the eight day after injury presented an active fibroblast (Fb) with short plasma membrane processes, large nucleus, considerable amount of free ribosomes, polysomes and expanded rough endoplasmic reticulum. n-nucleus, col-collagen. Bar 1 μm.

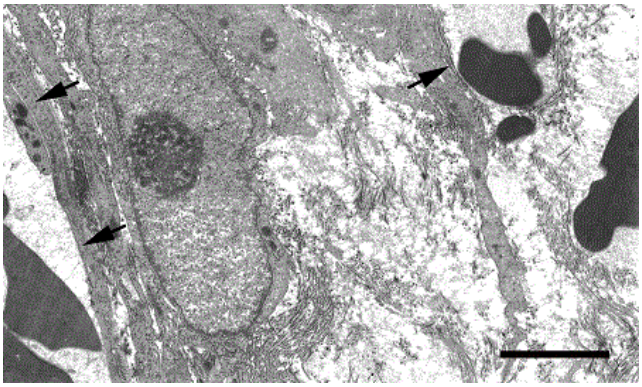

Figure 6: Transmission electron microscopy of the epiligament at the eight day after injury presented intensive angiogenesis with increased number of blood vessels (arrows) in the EL. Bar 1 μm.

Currently, there are only few studies that describe the morphological features of the EL of collateral ligaments of the knee joint in different animal models [4,5,8,10,13,15,26,27,29,30]. According to Lo et al. [13], it is possible EL to be the major source of cells engaged in the ligament scars formation during ligament healing. The process of ligament healing includes differentiation, phagocytosis, and collagen synthesis by the EL cells [13]. It is known that the fibroblasts are mobile cells, which migrate from EL to the healing ligament [8,30]. Chowdhury et al. [4] states that the EL cells closely bear a resemblance to the fibroblastic cells which compose ligament scar tissue. According to Chamberlain et al. [31] researches the ligament injury stimulates release of different cell types in the EL, including neutrophils and mitotic cells up to the 5th day after injury. There also were detected circulating macrophages, resident macrophages, T lymphocytes, hematopoietic cells, vascular endothelial growth factor with crest between 5 to 9 days post injury in the EL. Once localized in the ligament the cells and blood vessels in the EL

body proliferate and migrate. The process of creeping substitution by developing granulation tissue results in localization of cells from the healing region and into the healing edges [31]. The presence of EL cells within the ligament leads to a number of other conclusions [13]. The EL cells might be involved in differentiation, phagocytosis and collagen synthesis, and thus are involved in ligament healing process [13]. The study of Georgiev et al. [15] showed that on the eight day after injury the scar regions were characterized hyper-cellular and showed intensive angiogenesis (Figure 4). Migration of numerous cells in the deep part of the EL substance in the endoligament enveloping the collagen fibers of the ligament was registered. In unoperated animals there was relatively small number of cells in the EL presented near the ligament substance. By TEM observations it was found that there are active fibroblasts with short plasma membrane processes. Their large nuclei were with enormous prominent nucleolus, which is typical feature of actively synthesizing proteins cells. There was a considerable amount of free ribosomes, polysomes, expanded rough endoplasmic reticulum in the cytoplasm, which also refers to active protein synthesis (Figure 5). High incidence of lysosomes in fibroblasts of injured animals, in contrast to controls shows their increased phagocytic activity. High amounts of spherical mitochondria that are characteristic of a more intense metabolic activity was detected, which is opposite to uninjured animals. Intensive angiogenesis presented with increasing number of blood vessels in the EL substance is a manifestation of the late inflammation and early proliferative phase (Figure 6). Single or rarely small groups of collagen fibers arranged chaotically, did not reveal a well-presented recovery of the EL. Well-presented immunostaining for collagen type I and III (current results, Figure 7) and weak immunopositive reaction for collagen type V was detected at 8 day after healing throughout the MCL EL scar [10,30].

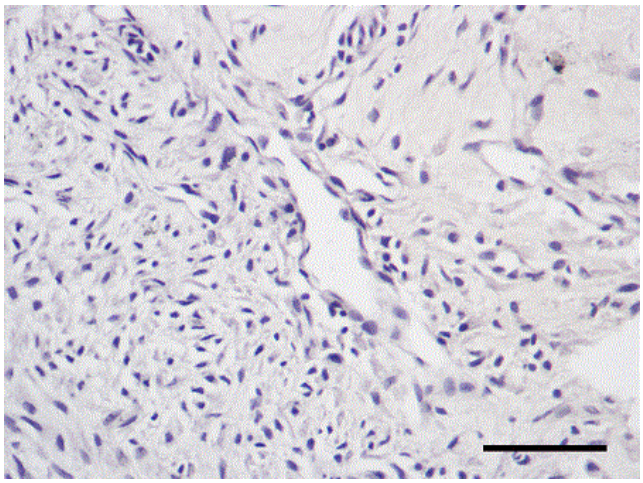

Figure 7: Immunohistochemical localization of collagen type III on the 8th day after injury. Bar 50 μm.

Light microscopy research on the sixteenth day after injury showed similar features as in previous period, but the granulation margins in the EL were less distinct and the area of scar formation appeared to be more organized (Figure 8). The deeper layer of the EL was also hypercellular, unlike controls and there also was migration of these cells in the endoligament covering the collagen fibres of the ligament. The fibroblasts in the scar area were also with large nuclei and plentiful rough endoplasmic reticulum as in previous period (Figure 9).

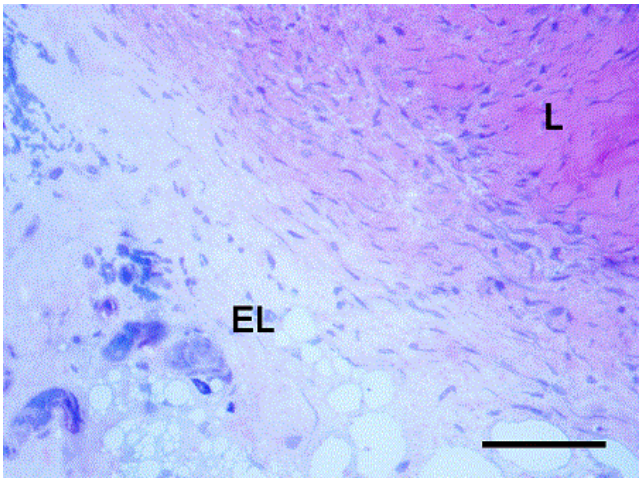

Figure 8: Epiligament (EL) – ligament (L) at the sixteenth day after injury: The light-microscopic photos, but on the ligament-epiligament area of scar formation appeared to be more organized. H&E. Bar 50 μm.

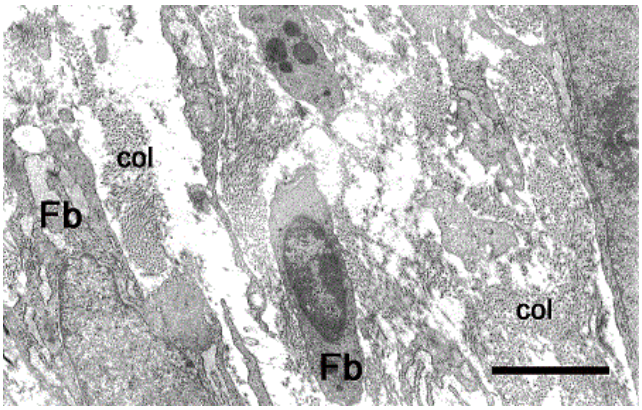

Figure 9: Transmission electron microscopy of the epiligament at the sixteenth day after injury presented fibroblasts with large nuclei and plentiful rough endoplasmic reticulum, although the number of lysosomes and mitochondria decreased. Bar 2 μm.

Although the number of lysosomes and mitochondria decreased, it was yet higher than controls. The described features point less phagocytic activity and less fibroblasts activation. In the zone of regeneration of the EL were detected single or clusters of fat cells that were a new packing material for EL tissue. Their shape was irregular and they varied in size compared to un-operated animals in which the adipose cells were single with spherical or polyhedral shape and were closely packed. In the remodeling phase the number of blood vessels in the EL tissue decreased. In this period the collagen fibers were organized in bundles with different orientations and damaged collagen fibers amongst them, but were more regular than in the previous period. Well-presented immunostaining for collagen type I and III (current results, there is no significant difference from the previous

period, Figure 10) and weak immunopositive reaction for collagen type V was detected at 16th day after healing throughout the MCL EL scar [10,30].

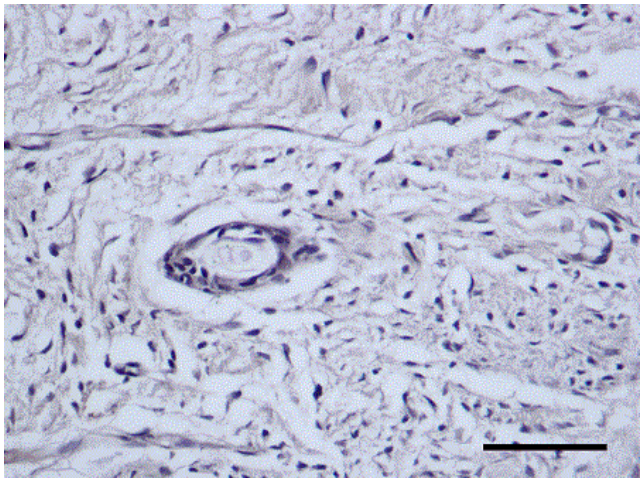

Figure 10: Immunohistochemical localization of collagen type III on the 16th day after injury. Bar 50 μm.

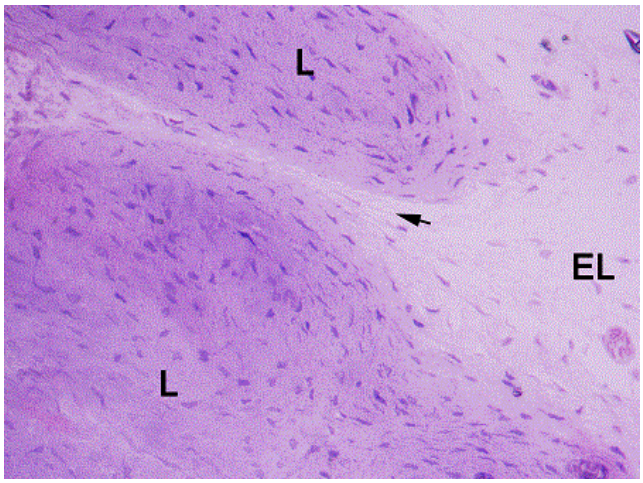

Figure 11: Epiligament (EL) – ligament (L) at the thirtieth day after injury: The EL tissue was composed of many fibroblasts and reduced number of vascular network. Endoligament enveloping the collagen fibers (black arrow). H&E. Bar 50 μm.

The healing process advanced and cells of the EL infiltrate most of the ligament scar, while collagen disorganization subsided on the thirtieth day after injury. The EL tissue was composed of fibroblasts, adipocytes, mast cells and reduced number of vascular network, but not fully restored, and quite similar to controls (Figure 11). The cells in the depth of the EL decreased in number like in controls. Similar to controls, transmission electron microscopy (TEM) presented mostly single lysosomes in the EL's fibroblasts. There were fibroblast with numerous lysosomes and single fibers with damaged characteristics seen incidentally, that presented the incomplete restoration of the MCL EL tissue (Figure 12). However, the expression of collagen type I, III (current results, less than the previous period, (Figure 13) and V on the 30th was similar to controls [10,30].

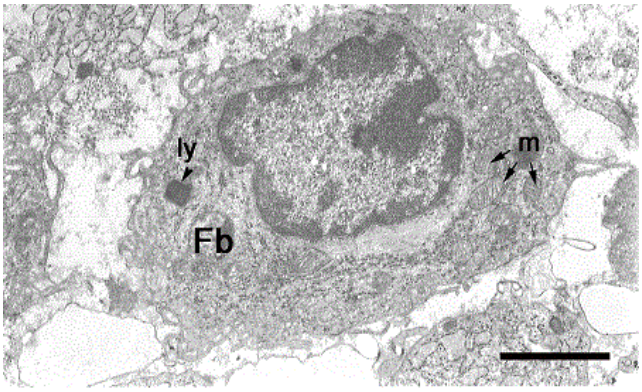

Figure 12: Transmission electron microscopy of the epiligament at the thirtieth day after injury presented a fibroblast (Fb) with mitochondria (m), ribosomes, lysosomes (ly) and single fibers with damaged characteristics seen incidentally. It presented the incomplete restoration of the EL tissue. Bar 2 μm.

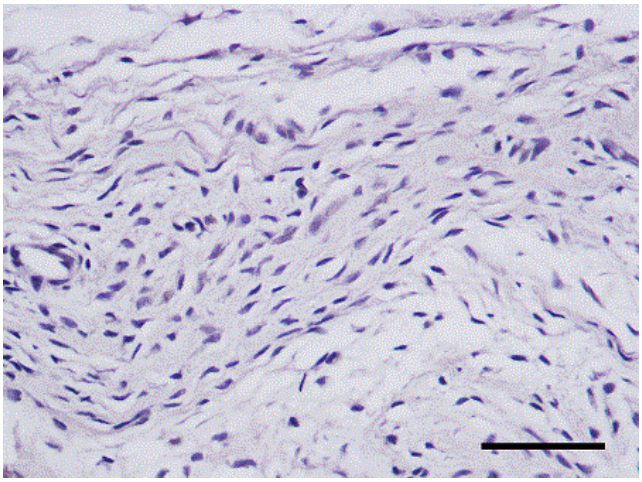

Figure 13: Immunohistochemical localization of collagen type III on the 30th day after injury. Bar 50 μm.

In conclusion, the EL of the collateral ligaments of the knee is quite a different structure from the ligament. It is composed of numerous fibroblasts, fibrocytes, adipocytes, and neuro-vascular bundles. As presented, the EL is the main source of fibroblasts, progenitor cells and blood vessels that proliferated and infiltrated within the ligament body via the endoligament during ligament healing. The fibroblasts in the EL are not static cells. They synthesize type I, III and V collagens, whose presence in the EL may be important in understanding the different parameters in evaluation of ligament healing.

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References

1. Amiel D, Frank C, Harwood F, Fronek J, Akeson W (1984) Tendons and ligaments: a morphological and biochemical comparison. J Orthop Res 1: 257-265.
2. Amiel D, Billings E, Akeson WH (1990) Ligament structure, chemistry and physiology: Knee Ligaments: Structure, Function, Injury and Repair. (1stedn), Raven Press, New York.
3. Arnoczky SP, Matyas JR, Buckwalter JA, Amiel D (1993) Anatomy of the anterior cruciate ligament: The Anterior Cruciate Ligament: Current and Future Concepts (1 stedn), Raven Press, New York.
4. Chowdhury P, Matyas JR, Frank CB (1991) The "epiligament" of the rabbit medial collateral ligament: a quantitative morphological study. Connect Tissue Res 27: 33-50.
5. Bray RC, Fisher AW, Frank CB (1990) Fine vascular anatomy of adult rabbit knee ligaments. J Anat 172: 69-79.
6. Georgiev GP, Vidinov NK (2009) Electron and light microscopic study of the epiligament of the lateral collateral ligament in a rat knee joint during early postnatal development. J Biomed Clin Res 2: 166-168.
7. Georgiev GP, Vidinov NK (2009) Investigation of the epiligament morphology of the lateral collateral ligament during postnatal development in a rat knee model. Compt rend Acad bulg Sci 62: 1473-1478.
8. Georgiev GP, Vidinov NK (2009) Epiligament changes after injury of the knee lateral collateral ligament in rat. J Biomed Clin Res 2: 96-98.
9. Georgiev GP, Landzhov B, Dimitrova IN, Slavchev S, Malinova L et al. (2015) Light microscopic and immunohistochemical study of the medial collateral ligament epiligament in rat knee. Compt rend Acad bulg Sci 68: 95-100.
10. Georgiev GP, Landzhov B, Dimitrova IN, Slavchev S, Malinova L, et al. (2015) Immunohistochemical study during early healing of the medial collateral ligament epiligament in rat knee model. Compt rend Acad bulg Sci .68: 655-660.
11. Junqueira LC, Carneiro J, Kelley RO (1998) Basic Histology. Connective tissue. (9thedn), Lange Medical Books/McGraw-Hill, New York – Toronto.
12. Lo IK, Ou Y, Rattner JP, Hart DA, Marchuk LL, et al. (2002) The cellular networks of normal ovine medial collateral and anterior cruciate ligaments are not accurately recapitulated in scar tissue. J Anat 200: 283-296.
13. Lo IK, Marchuk LL, Leatherbarrow KE, Frank CB, Hart DA (2004) Collagen fibrillogenesis and mRNA levels in the maturing rabbit medial collateral ligament and patellar tendon. Connect Tissue Res 45: 11-22.
14. Bray RC, Rangayyan RM, Frank CB (1996) Normal and healing ligament vascularity: a quantitative histological assessment in the adult rabbit medial collateral ligament. J Anat 188 : 87-95.
15. Georgiev GP, Vidinov NK, Kinov PS (2010) Histological and ultrastructural evaluation of the early healing of the lateral collateral ligament epiligament tissue in a rat knee model. BMC Musculoskelet Disord 11: 117.
16. Woo SL, Abramowitch SD, Kilger R, Liang R (2006) Biomechanics of knee ligaments: injury, healing, and repair. J Biomech 39: 1-20.
17. Yang L, Tsai CM, Hsieh AH, Lin VS, Akeson WH, et al. (1999) Adhesion strength differential of human ligament fibroblasts to collagen types I and III. J Orthop Res 17: 755-762.
18. Niyibizi C, Kavalkovich K, Yamaji T, Woo SL (2000) Type V collagen is increased during rabbit medial collateral ligament healing. Knee Surg Sports Traumatol Arthrosc 8: 281-285.
19. Liu SH, Yang RS, al-Shaikh R, Lane JM (1995) Collagen in tendon, ligament, and bone healing. A current review. Clin Orthop Relat Res : 265-278.
20. Breuls RG, Klumpers DD, Everts V, Smit TH (2009) Collagen type V modulates fibroblast behavior dependent on substrate stiffness. Biochem Biophys Res Commun 380: 425-429.
21. Andresen JL, Ledet T, Hager H, Josephsen K, Ehlers N (2000) The influence of corneal stromal matrix proteins on the migration of human corneal fibroblasts. Exp Eye Res 71: 33-43.
22. Miltner JL, Hu CH (1933) Experimental reproduction of joint sprains. Proc Soc Exp Biol Med 30: 883–884.
23. Frank C, Woo SL, Amiel D, Harwood F, Gomez M, et al. (1983) Medial collateral ligament healing. A multidisciplinary assessment in rabbits. Am J Sports Med 11: 379-389.
24. Frank CB, Hart DA, Shrive NG (1999) Molecular biology and biomechanics of normal and healing ligaments--a review. Osteoarthritis Cartilage 7: 130-140.
25. Frank C, Shrive N, Hiraoka H, Nakamura N, Kaneda Y, et al. (1999) Optimisation of the biology of soft tissue repair. J Sci Med Sport 2: 190-210.
26. Eng K, Rangayyan RM, Bray RC, Frank CB, Anscomb L, et al. (1992) Quantitative analysis of the fine vascular anatomy of articular ligaments. IEEE Trans Biomed Eng 39: 296-306.
27. Bray RC, Salo PT, Lo IK, Ackermann P, Rattner JB, et al. (2005). Normal ligament structure, physiology and function. Sports Med Arthrosc Rev 13: 127–135.
28. Hauser RA, Erin E, Dolan RN (2011). Ligament Injury and Healing: An Overview of Current Clinical Concepts. J Prolother 3: 836–846.
29. Chamberlain CS, Crowley E, Vanderby R (2009) The spatio-temporal dynamics of ligament healing. Wound Repair Regen 17: 206-215.
30. Georgiev GP, Kinov P, Rashev P, Sapundzhiev E, Vidinov NK (2010) Changes in the distribution of fibrillar collagens during early healing of the lateral collateral ligament epiligament tissue in rat knee model. Compt rend Acad bulg Sci 63: 761-766.

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