

Ultrastructural Assessment of the Anterolateral Ligament

Andrea Redler,^{*†‡} MD, PhD, Selenia Miglietta,[‡] PhD, Edoardo Monaco,[†] MD, Roberto Matassa,[‡] PhD, Michela Relucanti,[‡] PhD, Matthew Daggett,[§] DO, Andrea Ferretti,[†] MD, and Giuseppe Familiari,[‡] MD

Investigation performed at the University of Rome Sapienza, Rome, Italy

Background: The anterolateral ligament (ALL) has been identified as a structure on the lateral side of the knee, but debate exists regarding whether it is a capsular thickening or a ligament.

Hypothesis: A detailed ultrastructural characterization of the ALL and its ultrastructure collagen arrangement will reveal it more closely resembles ligamentous tissue than joint capsule.

Study Design: Descriptive laboratory study.

Methods: Eight paired knee samples from 4 fresh-frozen male cadavers were used for this study. Samples were harvested from the ALL, the joint capsule, and the medial collateral ligament (MCL). All samples were evaluated with light microscopy (LM), transmission electron microscopy (TEM), and variable pressure scanning electron microscopy (VP-SEM). With LM, the 3 tissues were analyzed and their morphology described. With TEM, the ultrastructure and collagen characteristics were quantified and compared among specimens. Then, the 3-dimensional characteristics were compared with VP-SEM.

Results: Ultrastructure analysis demonstrated similar morphology between the ALL and MCL, with significant differences in these 2 structures as compared with the joint capsule. On LM, the ALL and MCL were characterized by the presence of a dense collagen fiber oriented in the longitudinal and transversal directions of the fiber bundles, while the joint capsule was found to have a more disorganized architecture. On TEM, the collagen fibers of the ALL and MCL demonstrated similar ultrastructural morphology, with both having collagen fibers in parallel, longitudinal alignment. A quantitative analysis was also performed, with the mean (\pm SD) diameter of fibrils in the ALL and MCL being 80 ± 2.66 nm and 150 ± 3.35 nm, respectively (all $P < .001$). The VP-SEM highlighted that ALL and MCL morphology demonstrated arrangements of fiber bundles that are densely packed and organized, in contrast to the disorganized fibers of the joint capsule.

Conclusion: The ALL and MCL have comparable ultrastructures that are distinctly different from the joint capsule, as visualized on LM, TEM, and VP-SEM.

Clinical Relevance: The ALL should be considered a distinctive structure of the knee, although strictly connected to the surrounding capsule.

Keywords: anterolateral ligament; anterior cruciate ligament; electron microscopy; ultrastructure

With the initial description of the anterolateral ligament (ALL) by Claes et al,⁴ a renewed interest in the anterolateral structures of the knee has followed. Claes et al described the ALL as a distinct structure running from the lateral femoral epicondyle to the tibia, midway between the Gerdy tubercle and the fibular head. Subsequent anatomic and biomechanical studies have shown that the ALL is a distinct ligament in the human knee,¹⁹ with a specific function as a secondary restraint of the anterior cruciate ligament (ACL) in controlling tibial internal rotation and the pivot-shift phenomenon.^{22,23,26,27,30,34} However, much debate exists over the ALL. The precise anatomy and

function remain a topic of dispute, with identification of the ALL as a discrete ligament varying between 0% and 100%.^{5,7,10,17,28,36,39,43} Some researchers believe that the ALL is capsular thickening,^{3,11,39} while others believe it to be a ligamentous structure distinct from the surrounding joint capsule.^{8,31}

The morphological and ultrastructural microfeatures of collagen fibers have been investigated in other ligaments as their arrangement relates to their macroscopic mechanical function. Despite the plethora of anatomic and biomechanical investigations,¹¹ ultrastructural evaluation of the ALL has been confined to light microscopy (LM) descriptions.^{3,24,31,38,42} Among the limitations of LM are

The Orthopaedic Journal of Sports Medicine, 7(12), 2325967119887920

DOI: 10.1177/2325967119887920

© The Author(s) 2019

^{||}References 4, 5, 7, 17, 19, 22, 23, 26, 27, 29, 34, 36, 39, 43.

This open-access article is published and distributed under the Creative Commons Attribution - NonCommercial - No Derivatives License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits the noncommercial use, distribution, and reproduction of the article in any medium, provided the original author and source are credited. You may not alter, transform, or build upon this article without the permission of the Author(s). For article reuse guidelines, please visit SAGE's website at <http://www.sagepub.com/journals-permissions>.

limited resolution, magnification, and surface view. Electron microscopes offer a smooth range of magnifications and a greater depth of field compared with light microscopes. The purpose of our investigation was to provide a detailed ultrastructural characterization and comparison of the ALL, medial collateral ligament (MCL), and joint capsule through the use of LM, transmission electron microscopy (TEM), and variable pressure scanning electron microscopy (VP-SEM). We hypothesized that the ultrastructural arrangement of the ALL would be comparable with a ligament structure such as the MCL and different from the morphology of the joint capsule.

METHODS

Eight paired knee samples from 4 fresh-frozen male cadavers donated to a university anatomy program were used for the study. The cadavers were stored at -20°C and thawed to room temperature for 24 hours. The mean (\pm SD) age of specimens was 61.8 ± 6.4 years (range, 50-72 years). None of the donors had a history of lower extremity injury, cervical spine injury, immune disease, rheumatoid disease, or neurological disease. Additional exclusion criteria included signs of ligamentous injury, severe osteoarthritis seen at the time of dissection, bony abnormalities, or previous knee surgery. None of the specimens were excluded from the study. The cadavers remained fully intact, and no soft tissue was cut or removed from around the knee or adjacent joints, to most closely match a normal human knee.

A layer-by-layer dissection was performed with a 10-blade scalpel according to previously described techniques.^{8,41} The skin was removed circumferentially; the underlying adipose tissue was removed; and the superficial iliotibial band was incised. Careful dissection and documentation of each layer were then performed, until the joint was fully exposed (Figure 1).

The dissections were performed as Daggett et al⁶ previously described in a way to better visualize the ALL. A sample with an approximate size of 2×2 mm was harvested by the same surgeon (A.R.) from the middle part of the ALL. Other samples of the same shape and size were recovered from the joint capsule just anterior to the ALL and the MCL.

Sample Preparation for Light and Electron Microscopy

All samples were evaluated for LM, TEM, and VP-SEM at our university institute of anatomy, to investigate the

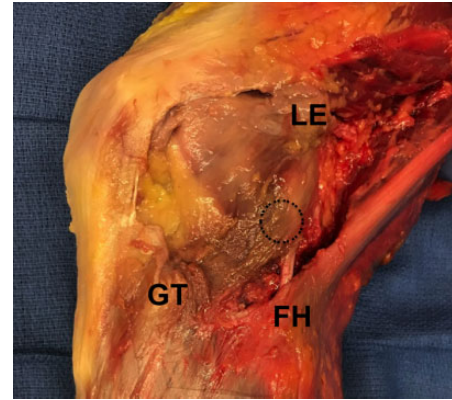


Figure 1. Lateral aspect of the knee shows a structure that attaches slightly posterior and proximal to the lateral epicondyle (LE) and courses distally to the tibia between the Gerdy tubercle (GT) and the fibular head (FH). This structure has previously been described as the deep capsulo-osseous layer of the iliotibial band and as the anterolateral ligament. The circle indicates the area of the ligament that was sampled, with an approximate size of 2×2 mm.

surface morphological and ultrastructural aspects. Sample fixation was performed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) solution. After fixation for 2 to 5 days at 4°C , the samples were rinsed in PBS, post-fixed with 1% osmium tetroxide (OsO_4 ; Agar Scientific) in PBS, and rinsed again in PBS.^{1,16,37}

Preparation for LM and TEM Observation

The samples were dehydrated in ascending series of ethanol, immersed in propylene oxide for solvent substitution, embedded in epoxy resin (Electron Microscopy Sciences), and sectioned by a Leica EM UC6 ultramicrotome. Sections $1 \mu\text{m}$ thick were stained with methylene blue, examined by LM (Zeiss Axioskop 40), and photographed with a digital camera (Leica DFC230). Ultrathin sections (60-80 nm) were cut on an ultramicrotome with a diamond knife, mounted on copper grids, and contrasted with lead citrate. They were examined and photographed with a Zeiss EM10 electron microscope operating at 80 kV.^{2,33,25} The quantitative evaluation was performed by the same blinded author (G.F.) with over 20 years of experience; 10 measurements of the fibril diameters were performed for each sample in 10 different points of view per field.

*Address correspondence to Andrea Redler, MD, PhD, Orthopaedic Unit and Kirk Kilgour Sports Injury Centre, S. Andrea Hospital, University of Rome Sapienza, Via di Grottarossa 1035-1039, 00189 Rome, Italy (email: andreadredler@gmail.com).

†Orthopaedic Unit and Kirk Kilgour Sports Injury Centre, S. Andrea Hospital, University of Rome Sapienza, Rome, Italy.

‡Department of Anatomy, Histology, Forensic Medicine and Orthopaedics, University of Rome Sapienza, Rome, Italy.

§Kansas City University of Medicine and Biosciences, Kansas City, Missouri, USA.

One or more of the authors has declared the following potential conflict of interest or source of funding: E.M. and A.F. have received consulting fees from Arthrex. M.D. has received consulting fees from Arthrex; grants from Apollo Surgical, Arthrex, Smith & Nephew, and Titan Surgical; and hospitality payments from DePuy and Zimmer Biomet. AOSSM checks author disclosures against the Open Payments Database (OPD). AOSSM has not conducted an independent investigation on the OPD and disclaims any liability or responsibility relating thereto.

Ethical approval for this study was obtained from the ethics committee of Sant'Andrea Hospital, University of Rome Sapienza.

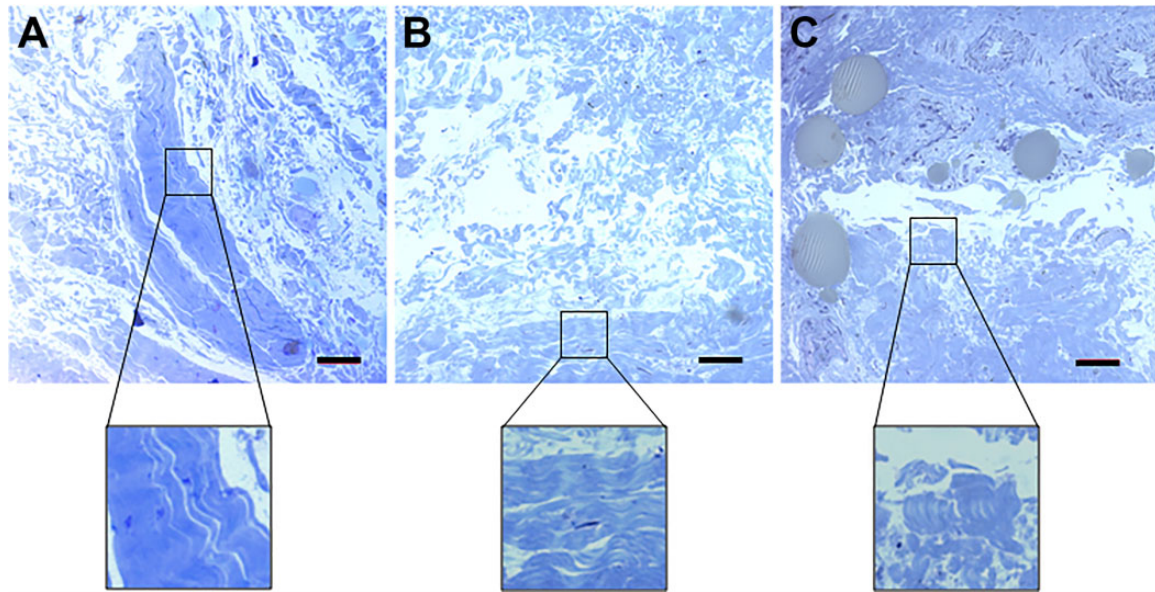


Figure 2. Histological images of the ALL and MCL ligaments and the joint capsule of connective tissues from fresh-frozen male cadavers. (A) ALL and (B) MCL collagen fiber sectioning. (C) Compacted collagen fibers with lipid drops (gray circle) of a joint capsule. Insets: identically sized magnified areas of dense collagen fiber oriented in the longitudinal and transversal directions of the corresponding figures. Scale bar = 60 μ m. ALL, anterolateral ligament; MCL, medial collateral ligament.

Preparation for VP-SEM Observation

To observe the native structure of the isolated bundle fibers through VP-SEM, the samples were kept in 2.5% glutaraldehyde for 48 hours and then prepared according to the protocol previously described by Ohtani et al³²; this method allows one to remove the surrounding extracellular matrix without affecting the collagen structure. This allows for visualization of the native morphology of exposed collagen fibers.

We observed our samples with VP-SEM (Hitachi SU-3500) operating at 5 kV with variable pressure at 20 Pa; we set these operating conditions to avoid artifact formations due to the effects of mechanical pressure and electron beam damage.

RESULTS

Light Microscopy

Histological analysis demonstrated similar morphology features between ALL and MCL sectioning, but clear differences were evident in comparison with the knee capsule (Figure 2). The ALL and MCL were characterized by the presence of a dense collagen fiber oriented in longitudinal and transverse directions of the fiber bundles (Figure 2, A and B). The LM observations of the sections exhibited similar waveform arrangement of collagen fibers for the ALL and MCL. The sectioning images for both structures also demonstrated areas containing compact tissue rich in elastin separated by wide interfiber spaces. The joint capsule demonstrated distinctly different characteristics. A more disorganized architecture was present, characterized

by the compacted collagen microislands, lipids, connective tissue, and neurovascular bundles. In addition, the organized collagen matrix seen in the ALL and MCL was distinctly absent in the joint capsule (Figure 2C).

Transmission Electron Microscopy

For each of the samples (MCLs, ALLs), 5 TEM microphotographs at a final magnification of 16,800 \times were randomly chosen and submitted for morphometric analysis.

The collagen fibers of the ALL and MCL demonstrated similar ultrastructural morphology on electron microscopy (Figure 3). Both collagen fibers had a parallel alignment orientated mainly longitudinally (Figure 3, A and B). One slight difference between these structures was the dimension of the single fibrils of collagen. The mean diameter of fibrils in the ALL was 80 ± 2.66 nm, while in the MCL it was 150 ± 3.35 nm (Table 1) ($P < .001$). Significant structural differences of the tissue of the joint capsule as compared with the ligaments were observed (Figure 3C). The joint capsule demonstrated a higher amount of fibroblast cells with large nuclei transversally oriented, with a few disordered collagen fibrils dispersed throughout the tissue. The synovocyte cells (Figure 3D) in particular had the typical features of the cellular lining in the internal surface belonging to the joint capsule. Synovocyte cells with evident chromatin with the nucleus, several vacuoles outside, and developed endoplasmic reticulum were observed.

Variable Pressure Scanning Electron Microscopy

Surface morphological investigations demonstrated the ultrasonic structure of the collagen fibers alone, per the

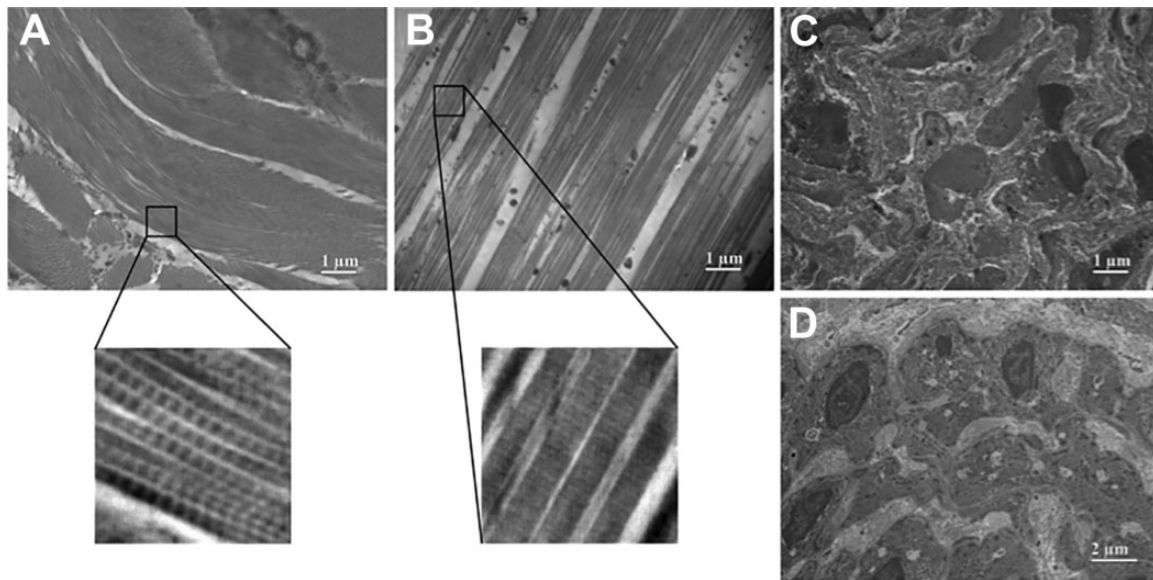


Figure 3. Ultrastructural morphology of the ALL, MCL, and joint capsule. (A) ALL collagen and (B) MCL collagen fibers. Insets: identically sized magnified areas of aligned fibrils of the corresponding figures. (C) Tissue of the joint capsule. (D) Synovioocyte cells of the joint capsule. ALL, anterolateral ligament; MCL, medial collateral ligament.

TABLE 1
Quantitative Analysis of the Fibrils
in the MCL, ALL, and Joint Capsule^a

Sample	Diameter, nm, Mean ± SD		
	MCL	ALL	Joint Capsule
1	148 ± 2.44	77 ± 1.44	39.5 ± 6.72
2	152 ± 1.52	81 ± 1.83	40 ± 6
3	148 ± 2.60	83 ± 1.24	39.9 ± 6.43
4	147 ± 2.24	79 ± 2.16	39.4 ± 6.9
5	150 ± 2.13	82 ± 1.47	39.5 ± 6.25
6	150 ± 2.58	80 ± 1.86	39.9 ± 6.11
7	147 ± 1.86	79 ± 1.37	39.1 ± 6.8
8	149 ± 2.98	80 ± 1.51	40 ± 6.35

^aALL, anterolateral ligament; MCL, medial collateral ligament.

sample preparation protocol of Ohtani et al.³² By cell maceration of the extracellular matrix components, we were able to observe the real 3-dimensional skeletal network of the bundle fibers (Figure 4). The ALL demonstrated wavy and aligned arrangements of fiber bundles that were densely packed (Figure 4A). The MCL bundle fibers were also densely packed with regular and parallel shape in aligned bundles, but in contrast they did not have a waveform arrangement (Figure 4B). A qualitative observation of VP-SEM images demonstrated that the fiber dimension of the ALL was smaller than that of the MCL fibers, in agreement with the TEM results.

While significant similarities exist between these structures, the joint capsule demonstrated a completely different organization of fibers (Figure 4C). The 3-dimensional surface morphology demonstrated disordered fibers arranged in a radially and curled manner, forming microcavities and

a fibrous network and corresponding to the surface of the synovial membrane observed in Figure 4D.

DISCUSSION

Previous studies have used TEM and VP-SEM to classify and differentiate capsule and ligamentous ultrastructures.^{12,14,45} TEM and VP-SEM high-resolution power allows visualization of collagen bundle arrangement and thickness to morphological parameters that help to clearly distinguish between capsule and ligament ultrastructure. Our findings comparing the ALL, MCL, and joint capsule confirmed that, based on ultrastructure analysis, the ALL is a ligament.

Observation of LM sections showed that the ALL and MCL have a common structure, with compact tissue slightly vascularized and rich in elastin, further characterized by the presence of dense collagen fiber organized in wavy parallel bundles.⁴⁵ Several previous studies have performed histologic analysis of the ALL.^{3,17,31,38,41} Vincent et al⁴² noted wavy parallel dense collagenous fibers suggestive of ligamentous tissue within the ALL isolated from cadavers. Helito et al¹⁷ analyzed 20 specimens and showed that the ALL has typical histologic characteristics seen in ligamentous structures described as dense connective tissue with arranged fibers. Moreover, Catherine et al³ characterized the anatomic properties of the ALL, describing the ALL fibers at LM as being similar to those of the ACL. LM results reported in a 2017 study by Smeets et al³⁸ demonstrated that the ALL was histologically similar to the inferior glenohumeral ligament and different from the knee capsule.

In a 2018 study, Neri et al³¹ evaluated the femoral insertion of the ALL and its relationship with the lateral

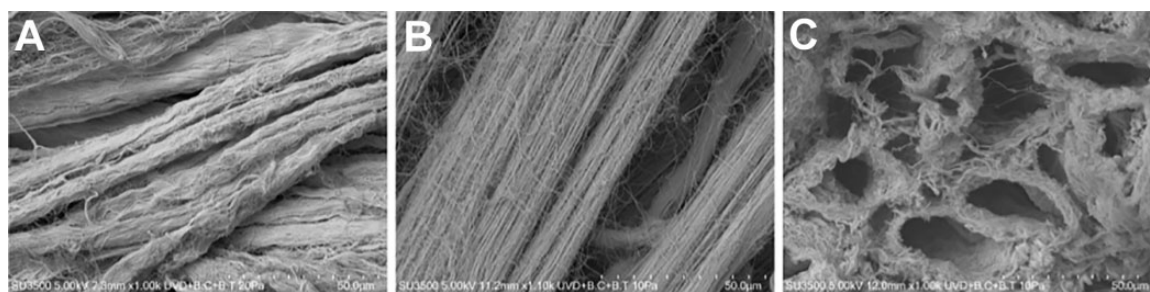


Figure 4. Three-dimensional surface morphology of the ALL, MCL, and joint capsule. (A) ALL collagen and (B) MCL bundle fibers. (C) Microcavities of the joint capsule. ALL, anterolateral ligament; MCL, medial collateral ligament.

collateral ligament. The authors demonstrated that the ALL has all the histological characteristics of a ligamentous structure and confirmed that the ALL can be considered a real and distinct ligament. Daggett et al⁸ demonstrated the ALL to be a histologically distinct structure in en block dissection analysis of the anterolateral knee. Dombrowski et al,¹¹ in their histological analysis of the knee joint, described that lateral capsular thickening demonstrated a distinct transition from loose connective tissue resembling a capsule to an organized structure resembling a ligamentous tissue.

Observation of the ultrastructural features of the ALL by TEM and VP-SEM demonstrated the existence of a waveform arrangement of aligned fibrils forming wavy collagen fiber bundles crossing and intertwining each other yet oriented along the main axis of the ligament. Similarly, the MCL demonstrated straight parallel fibrils forming the bundle fibers and being densely packed and ordered with similar longitudinal direction. This behavior of the MCL collagen fibers was also observed by Zaffagnini et al⁴⁵ in the human knee. However, the outer part of the posterior intermediate region of the MCL was found to have intertwining and crossing arrangements of the fibrils in a wavy pattern, recognized as micron crimps. Waveform behavior of collagen fibers in tendons and ligaments observed through electron microscopy techniques has been described as a multifascicular structure with collagen fiber bundles cross-connecting to each other.^{19,41,44} In the current study, collagen fibers were running parallel and densely packed, interrupted only by periodic areas waves in the presence of micron crimps observed in the MCL.

These morphological features found in tendons and ligaments play a crucial role in preventing damage and disconnection of the collagen fibers because their change direction works as a shock absorber system.^{9,12,13,15,18,21} The prominent waveform configuration of collagen fiber bundles of the ALL, as observed in our findings, further supports its function as a ligament, avoiding tensile stress during tibial intrarotation owing to the elastic properties of the ultrastructure of the tissue.

Interestingly, the anatomic ultrastructure of the single ALL fibril clearly demonstrated a slightly smaller diameter than that of the MCL fibrils observed at the nanoscopic scale through TEM. We hypothesize that the fibers were smaller in the ALL because of the functional differences

of the 2 structures; the ALL is a secondary stabilizer to the ACL in resisting anterior tibial translation and internal tibial rotation and in preventing the knee pivot-shift phenomenon. The MCL is important to maintain primary medial knee stability and provides a primary and secondary role in providing stabilization against abnormal valgus motion, external/internal rotation, and anterior/posterior translation in the knee. Thus, the mechanical strength must necessarily be greater in the MCL than the ALL.

Our morphometric analysis indicated that the ALL fibrils were narrow and unimodal in size. The MCL has a multimodal distribution depending on the portion, region, and division of the MCL, as noted by Zaffagnini et al,⁴⁵ and Strocchi et al⁴⁰ demonstrated a bidimensional arrangement in the ACL. In the current study, we did not see collagen fibers of a different nature, shape, and size in the MCL, probably because the sample size for the electron microscopy technique was not big enough to have a surface to analyze. Therefore, the analyzed areas gave homogeneous results.

Although there were slight differences in the diameter of the fibrils between the ALL and the MCL, they shared similar characteristics in that they demonstrated parallel wavy arrangements running longitudinally to the ligament course. As such, this assembled morphology featured an intrinsic mechanical property of the fiber bundles that supports the nomenclature labeling the ALL a ligament. These wavy fiber bundles with unimodal size distribution appeared in all cadaveric samples and was confirmed for the first time by the combined use of scanning and TEM. The histological and ultrastructural appearances of the ACL differ from the other ligaments and tendons, with 2 types of fibrils: the first with a variable diameter and irregular outline, the second with a uniform diameter and smooth profile. However, similarities were found between the ALL and the ACL, especially from a histological and cellular point of view.⁴⁰ The morphology of the ALL was also remarkably similar to that of the inferior glenohumeral ligament, with data from several studies supporting the hypothesis that the ALL and inferior glenohumeral ligament are comparable structures with similar ultrastructural and biomechanical properties.³⁸

Our findings regarding the knee capsule are consistent with previous findings.^{20,35} The knee capsule demonstrated a more disorganized architecture characterized by the

presence of the collagen microislands, lipids, connective tissue, and neurovascular bundles. Therefore, the joint capsule demonstrated distinctly different morphostructural properties when compared with the ligaments of the ALL and MCL.

Limitations

This study has some limitations. It was a cadaveric study with a limited number of samples, and the mean age of the cadavers may not represent the typical population undergoing knee ligament reconstruction. We had a limited sample size; other studies have shown a difference in MCL morphology in different areas⁴⁵ of the ligament, and perhaps the same is true for ALL. We did not perform biomechanical analysis of these structures, and further studies should be performed to better understand the similarities and differences between these structures.

CONCLUSION

To the best of our knowledge, this is the first study describing the ALL ultrastructurally. Our findings indicate that the ALL and MCL have comparable ultrastructures that are distinctly different from the joint capsule, as visualized on LM, TEM, and VP-SEM.

REFERENCES

- Agha-Rahimi A, Khalili MA, Nottola SA, et al. Cryoprotectant-free vitrification of human spermatozoa in new artificial seminal fluid. *Andrology*. 2016;4(6):1037-1044.
- Amici A, Caracciolo G, Digiacoimo L, et al. In vivo protein corona patterns of lipid nanoparticles. *RSC Advances*. 2017;7:1137-1145.
- Caterine S, Litchfield R, Johnson M, et al. A cadaveric study of the anterolateral ligament: re-introducing the lateral capsular ligament. *Knee Surg Sports Traumatol Arthrosc*. 2015;23:3186-3195.
- Claes S, Vereecke E, Maes M, et al. Anatomy of the anterolateral ligament of the knee. *J Anat*. 2013;223(4):321-328.
- Coquart B, Le Corroller T, Laurent PE, et al. Anterolateral ligament of the knee: myth or reality? *Surg Radiol Anat*. 2016;38(8):955-962.
- Daggett M, Busch K, Sonnery-Cottet B. Surgical dissection of the anterolateral ligament. *Arthrosc Tech*. 2016;5(1):e185-e188.
- Daggett M, Ockuly AC, Cullen M, et al. Femoral origin of the anterolateral ligament: an anatomic analysis. *Arthroscopy*. 2016;32(5):835-841.
- Daggett M, Stephenson C, Dobson J, et al. Anatomic and histological study of the anterolateral aspect of the knee: a SANTI Group investigation. *Orthop J Sports Med*. 2018;6(10):2325967118799970.
- Danylchuk KD, Finlay JB, Krcek JP. Microstructural organization of human and bovine cruciate ligaments. *Clin Orthop Rel Res*. 1978;131:294-298.
- De Carli A, Monaco E, Mazza D, et al. Assessment of the anterolateral ligament of the knee by magnetic resonance imaging. *Joints*. 2018;6(3):153-156.
- Dombrowski ME, Costello JM, Ohashi B, et al. Macroscopic anatomical, histological and magnetic resonance imaging correlation of the lateral capsule of the knee. *Knee Surg Sports Traumatol Arthrosc*. 2016;24(9):2854-2860.
- Franchi M, Fini M, Quaranta M, et al. Crimp morphology in relaxed and stretched rat Achilles tendon. *J Anat*. 2007;210:1-7.
- Franchi M, Quaranta M, Macciocca M, et al. Collagen fibre arrangement and functional crimping pattern of the medial collateral ligament in the rat knee. *Knee Surg Sports Traumatol Arthrosc*. 2010;18:1671-1678.
- Franchi M, Quaranta M, Macciocca M, et al. Structure relates to elastic recoil and functional role in quadriceps tendon and patellar ligament. *Micron*. 2009;40:370-377.
- Frank CB, Hart DA, Shrive NG. Molecular biology and biomechanics of normal and healing ligaments—a review. *Osteoarthritis Cartilage*. 1999;7:130-140.
- Halvaei I, Khalili MA, Esfandiari N, et al. Ultrastructure of cytoplasmic fragments in human cleavage stage embryos. *J Assist Reprod Genet*. 2016;33(12):1677-1684.
- Helito CP, Demange MK, Bonadio MB, et al. Anatomy and histology of the knee anterolateral ligament. *Orthop J Sports Med*. 2013;1(7):2325967113513546.
- Hurschler C, Provenzano PP, Vanderby R Jr. Scanning electron microscopic characterization of healing and normal rat ligament microstructure under slack and loaded conditions. *Connect Tissue Res*. 2003;44:59-68.
- Ingham SJM, de Carvalho RT, Martins CAQ, et al. Anterolateral ligament anatomy: a comparative anatomical study. *Knee Surg Sports Traumatol Arthrosc*. 2017;25(4):1048-1054.
- Iwanaga T, Shikichi M, Kitamura H, Yanase H, Nozawa-Inoue K. Morphology and functional roles of synoviocytes in the joint. *Arch Histol Cytol*. 2000;63(1):17-31.
- Jarvinen TA, Jarvinen TL, Kannus P, et al. Collagen fibres of the spontaneously ruptured human tendons display decreased thickness and crimp angle. *J Orthop Res*. 2004;22:1303-1309.
- Kittl C, El-Daou H, Athwal KK, et al. The role of the anterolateral structures and the ACL in controlling laxity of the intact and ACL-deficient knee. *Am J Sports Med*. 2016;44(2):345-354.
- Lutz C, Sonnery-Cottet B, Niglis L, et al. Behavior of the anterolateral structures of the knee during internal rotation. *Orthop Traumatol Surg Res*. 2015;101(5):523-528.
- Macchi V, Porzionato A, Morra A, et al. The anterolateral ligament of the knee: a radiologic and histotopographic study. *Surg Radiol Anat*. 2016;38(3):341-348.
- Matassa, Familiari G, Battaglione E, et al. Electron microscopy reveals layered architecture of individual gold nanoparticles self-anchored by fluorescence monomers. *Nanoscale*. 2016;8:18161-18169.
- Monaco E, Fabbri M, Mazza D, et al. The effect of sequential tearing of the anterior cruciate and anterolateral ligament on anterior translation and the pivot-shift phenomenon: a cadaveric study using navigation. *Arthroscopy*. 2018;34(4):1009-1014.
- Monaco E, Ferretti A, Labianca L, et al. Navigated knee kinematics after cutting of the ACL and its secondary restraint. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(5):870-877.
- Monaco E, Helito CP, Redler A, et al. Correlation between magnetic resonance imaging and surgical exploration of the anterolateral structures of the acute anterior cruciate ligament-injured knee. *Am J Sports Med*. 2019;47(5):1186-1193.
- Monaco E, Lanzetti RM, Fabbri M, et al. Anterolateral ligament reconstruction with autologous grafting: a biomechanical study. *Clin Biomech (Bristol, Avon)*. 2017;44:99-103.
- Musahl V, Getgood A, Neyret P, et al. Contributions of the anterolateral complex and the anterolateral ligament to rotatory knee stability in the setting of ACL injury: a roundtable discussion. *Knee Surg Sports Traumatol Arthrosc*. 2017;25(4):997-1008.
- Neri T, Dalcol P, Palpacuer F, et al. The anterolateral ligament is a distinct ligamentous structure: a histological explanation. *Knee*. 2018;25(3):360-366.
- Ohtani O, Ushiki T, Taguchi T, et al. Collagen fibrillar networks as skeletal frameworks: a demonstration by cell-maceration/scanning electron microscope method. *Arch Histol Cytol*. 1988;51(3):249-261.
- Palmerini MG, Belli M, Nottola SA, et al. Mancozeb impairs the ultrastructure of mouse granulosa cells in a dose-dependent manner. *J Reprod Dev*. 2018;64(1):75-82.
- Parsons EM, Gee AO, Spiekerman C, Cavanagh PR. The biomechanical function of the anterolateral ligament of the knee. *Am J Sports Med*. 2015;43(3):669-674.

35. Ralphs JR, Benjamin M. The joint capsule: structure, composition, ageing and disease. *J Anat.* 1994;184(pt 3): 503-509.
36. Shea KG, Polousky JD, Jacobs JC Jr, et al. The anterolateral ligament of the knee: an inconsistent finding in pediatric cadaveric specimens. *J Pediatr Orthop.* 2016;36(5):e51-e54.
37. Siciliano C, Bordin A, Ibrahim M, et al. The adipose tissue of origin influences the biological potential of human adipose stromal cells isolated from mediastinal and subcutaneous fat depots. *Stem Cell Res.* 2016;17(2):342-351.
38. Smeets K, Slane J, Scheys L, et al. The anterolateral ligament has similar biomechanical and histologic properties to the inferior glenohumeral ligament. *Arthroscopy.* 2017;33(5): 1028-1035.
39. Stijak L, Bumbaširević M, Radonjić V, et al. Anatomic description of the anterolateral ligament of the knee. *Knee Surg Sports Traumatol Arthrosc.* 2016;24(7):2083-2088.
40. Stocchi R, De Pasquale V, Gubellini P, et al. The human anterior cruciate ligament: histological and ultrastructural observations. *J Anat.* 1992;180:515-519.
41. Terry GC, Hughston JC, Norwood LA. The anatomy of the iliopatellar band and iliotibial tract. *Am J Sports Med.* 1986;14(1):39-45.
42. Vincent JP, Magnussen RA, Gezmez F, et al. The anterolateral ligament of the human knee: an anatomic and histologic study. *Knee Surg Sports Traumatol Arthrosc.* 2012;20(1):147-152.
43. Watanabe J, Suzuki D, Mizoguchi S, et al. The anterolateral ligament in a Japanese population: study on prevalence and morphology. *J Orthop Sci.* 2016;21(5):647-651.
44. Yahia LH, Garzon S. Structure on the capsular ligaments of the facet joints. *Ann Anat.* 1993;175:185-188.
45. Zaffagnini S, Marcheggiani Muccioli GM, et al. Collagen fibre and fibril ultrastructural arrangement of the superficial medial collateral ligament in the human knee. *Knee Surg Sports Traumatol Arthrosc.* 2015;23(12):3674-3682.