

Mechanical and structural tensile properties of the human Medial Patello-femoral Ligament

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Abstract

The medial patellofemoral ligament (MPFL) is considered the most important passive patellar stabilizer and acts 50-60% of the force of the medial soft-tissue which restrains the lateralization of the patella between 0° and 30°. In this work, 20 human knees have been tested to evaluate the mechanical properties of MPFL and to determine the structural behaviour of femur-MPFL-Patella complex (FMPC). Particular attention was given to maintain the anatomical orientation between the patella and MPFL and to the evaluation of the elongation during the mechanical tests. The ultimate stress of the isolated ligament was 16 ± 11 MPa, the ultimate strain was $24.3\% \pm 6.8\%$, the Young's Modulus was 116 ± 95 MPa and the strain energy density was 2.97 ± 1.69 MPa.

The ultimate load of the whole structure, FMPC, was 145 ± 68 N, the ultimate elongation was 9.5 ± 2.9 mm, the linear stiffness was 42.5 ± 10.2 N/mm and the absorbed energy was 818.8 ± 440.7 N-mm. The evaluation of mechanical and structural properties of MPFL is fundamental to understand its contribution as stabilizer and for the selection of repair and reconstruction methods.

1. Introduction

Patellar subluxation is a common disease. Annually, about 6 out of 100,000 in the general population suffers from primary patellar dislocation and more than 90% of the cases result in MPFL injury (Atkin et al., 2000 and Fithian et al., 2004). After the first episode of dislocation there is a 17% probability of recurrence and, after the second episode, 49% of the patients will experience further recurrences (Fisher et al., 2010). Surgical treatment is usually indicated when conservative treatment fails or in those cases where repeated recurrent subluxations occur (Servien et al., 2011).

The medial patello-femoral ligament (MPFL) seems to be the most important passive patellar stabilizer and it acts 50-60% of the force of the medial soft-tissue which restrains the lateralization of the patella between 0° and 30° (Cash et al., 1988; Cofield et al., 1977; Hawkins et al., 1986; Larsen et al., 1982; Mäenpää et al., 1997; Mountney et al., 2005; Bicos et al., 2007; Conlan et al., 1993; Desio et al., 1998; Hautamaa et al., 1998).

Nevertheless, some authors in cadaveric studies were unable to locate this ligament in some of the anatomical specimens after dissection (Amis et al., 2003; Reider et al., 1981) creating further debate concerning the MPFL's function or its existence.

In 1979, Warren and Mashall, dissecting 154 anatomical specimens, observed that the anatomy of the structures located anteriorly to the superficial collateral medial ligament and medially to the medial edge of the patella is extremely variable (Warren et al., 1979). Later studies have shown that the MPFL also varies in insertion, thickness and resistance. In the majority of cases it is palpable upon dissection and in a small percentage it is frail and thin (Nomura et al., 2005; Philippot et al., 2009; Baldwin et al., 2009).

The kinematics of the patello-femoral joint are sustained by static and dynamic factors that stabilize and center the patella within the trochlea. The static stabilizers do not change their size according to the movement phase. Dynamic stabilizers, however, adapt by varying their size and position according to the forces acting on the patella.

Biomechanical studies have shown that the main medial static stabilizer of the patella is the MPFL which together with the medial patello-meniscal ligament, the medial patello-tibial ligament and the medial retinaculum prevents the external subluxation of the patella (Panagiotopoulos et al., 2006). Indeed, the evaluation of mechanical behaviour of the MPFL is fundamental to understand its contribution as stabilizer.

(Burks et al., 1998) have examined the tensile behaviour of the MPFL finding a mean failure load of 209 N at 25 mm of displacement for failure of the MPFL during lateral patellar dislocation.

Similar results have been proposed by (Mountney et al., 2005). Also (Arendt et al., 2007) tested 12 cadaveric specimens and they found that the ultimate load was 145.6 N.

The differences between these results are related both to biologic variability and to experimental testing setup. From a biological point of view, (Woo et al., 1999) demonstrated that the differences in specimen age, species, skeletal maturation, anatomic location, and exercise or immobilization can affect the biomechanical properties of ligaments (Woo et al., 1999). On the other side, experimental testing factors, such as specimen, orientation and geometry, status of the insertions, temperature of the specimen during testing, and strain rate can also affect the biomechanical properties of these tissues. In particular, Kim et al. demonstrated that the orientation of the specimen during tensile testing has a significant effect on stiffness and failure modes of the porcine FMPC (Kim et al., 2014).

The objective of this study is the evaluation of the tensile behaviour of human MPFL distinguishing between mechanical properties of ligament substance and structural ones of femur-MPFL-patella complex (FMPC).

To fulfil this objective, we have to provide an experimental design which allows us to overcome the three issues above explained. The mechanical properties of the MPFL substance will be determined through uniaxial tensile test of the isolated ligament with standardized size and with normalized velocity, to obtain a stress-strain relation. In addition, particular attention was given to the method to evaluate the elongation, coupling data from the testing device with optical measurements based on markers placed on the specimen.

The anatomical orientation between the patella and ligament, which determines the correct load during the mechanical test on the FMPC, was guaranteed by a dedicated gripping system. In both

cases, particular attention was given to the method to evaluate the elongation, coupling data from the tensile testing device with optical measurements in case of MPFL characterization, and with measurements based on an infrared motion system in case of FMPC characterization.

2. Materials and Methods

2.1 Preparation of specimens

A total of 24 human cadaveric knees from 6 women and 8 men with a mean age of 75 ± 9 years were used in this experiment. None of these showed patellar instability, knee injuries, surgical procedures or arthritic deformations. The Nicola's Foundation Onlus Ethics Committee has given its approval for this study.

The cadavers were dissected after they have been stored for 24 hours at 4°C and then were preserved in a sterile gauze, sealed in a polyethylene bag, labelled and stored at -18°C. They were thawed at a temperature of 4°C when required.

Warren and Marshall's three-layer classification of the medial side of the knee was used to dissect the specimens (Warren et al., 1979).

All dissections started with a midline incision of the skin detaching it from the subcutaneous fascia. The joint capsule was accessed extending through a lateral incision at the vastus lateralis muscle extending laterally at the parapatellar side and at the lateral compartment of the tibia which was cut proximally to distally. The femur was tipped up detaching the muscle bundles. The isolated single muscles of the quadriceps were left inserted in their distal insertions and used as landmarks. The patella was rotated and the posteromedial capsule was opened from the inside, detaching and isolating the synovial capsule in the 3rd layer from the 2nd one. The fibers of the MPFL were identified by palpation and direct vision.

After, we proceeded from the external side of the dissection at Layer 1, paying particular attention to not damage the ligament, with blunt and anatomical forceps, because of the extreme adhesion between these two layers.

Then, the next step was the identification of the Medial Epicondyle (E), the Adductors Tubercle (AT), the insertion of Medial Collateral Ligament (MCL), the Magnus Adductor Muscle (MAM) and the femoral insertion of MPFL (Placella et al., 2014).

Finally, The MPFL was isolated such that it was the only connection between the patella and the femur (Fig. 1). The MPFL was found in all the examined knees.



Fig. 1 - MPFL dissected specimen

After dissection, the length, the thickness, the width and the cross-sectional areas of the MPFL at patellar insertion, mid-substance, and femoral insertion were measured using a digital caliper (accuracy of 0.02 mm and resolution of 0.01 mm) and a digital micrometer (accuracy of 0.01 mm and resolution of 0.01 mm). Before the tensile tests, the specimens were left in 37° saline bath for 30 min.

2.2 Uniaxial Tensile tests

Mechanical characterization can be divided into two subsequent steps: isolated ligament and FMPC tests.

2.2.1 Isolated ligament uniaxial tensile tests

A total of 12 isolated ligaments were tested; specimens were rectangular shaped with surgical scalpel so that the length-to-width aspect ratio (4:1) provided uniform tensile stress in the region where the strain was measured (Woo et al., 1976).

All the specimens had a constant cross-sectional area (CSA), it was measured with a digital caliper and we assumed it had a rectangular shape.

The isolated ligament was fixed with cyano-acrilate and sandpaper in a standard clamp and it was axially aligned to the 5kN load cell of an Instron 5965 materials-testing machine.

A custom 2D optical system was used to evaluate the elongation of the isolated ligament. The system consisted in a camera (CANON EOS 60D, mounting a camera lens EF-S 18-135 mm) and four markers which have been positioned and vertically aligned on four different areas to evaluate the ligament elongation and possible slippage (Fig. 2).

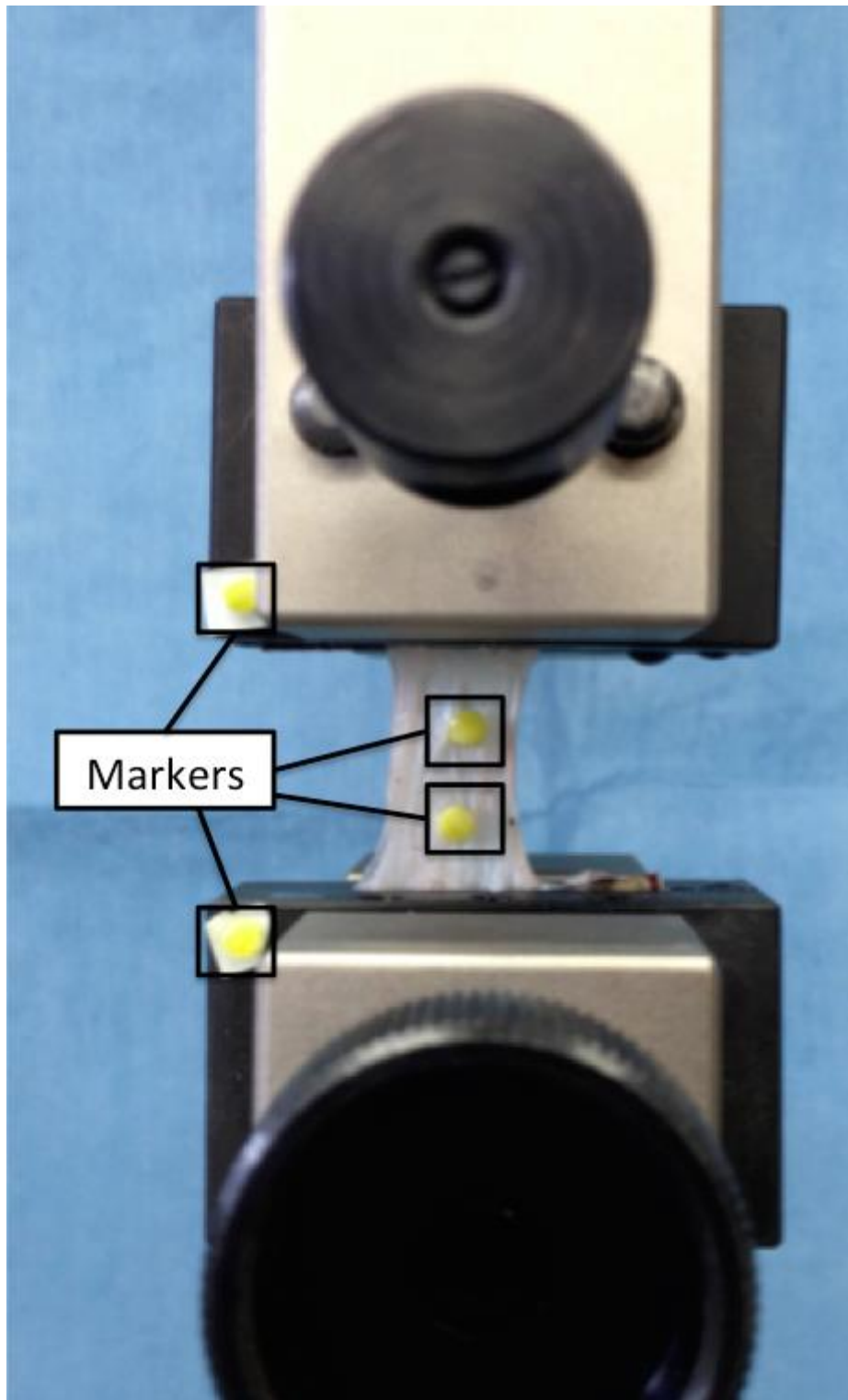


Fig. 2 – Isolated ligament tensile test setup. A custom 2D optical system (a camera and four markers positioned and vertically aligned on four different areas of the ligament) was used to evaluate the elongation and possible slippage.

The specimen was preconditioned by a series of ten cycles, to 3% of strain at a strain rate of 0.1%/s, in order to reduce tissue hysteresis. The ligament was then tested to failure at a strain rate

of 0.3%/s. With cross-sectional area and strain measurements, a stress–strain curve representing the mechanical properties of the ligamentous tissue was created (Woo et al., 1999). From the stress-strain curves, the following parameters, which define the mechanical properties of the MPFL, were obtained: Young’s Modulus (MPa), defined as the slope of the linear region of the stress-strain curve between 5% and 10% of strain, ultimate stress (MPa), ultimate strain (%) and strain energy density (MPa) at failure (Woo et al., 1999). The failure mode was also noted.

2.2.2 FMPC uniaxial tensile tests

Twelve FMPCs were tested. A custom designed mechanical frame was designed to align the FMPC specimen with the 5 kN load cell in order to obtain a correct uniaxial tensile test in anatomical position (Fig. 3).



Fig. 3 – FMPC tensile test set up. A specific mechanical frame was designed to align the FMPC specimen with the 5 kN load cell to obtain a correct uniaxial tensile test in anatomical position.

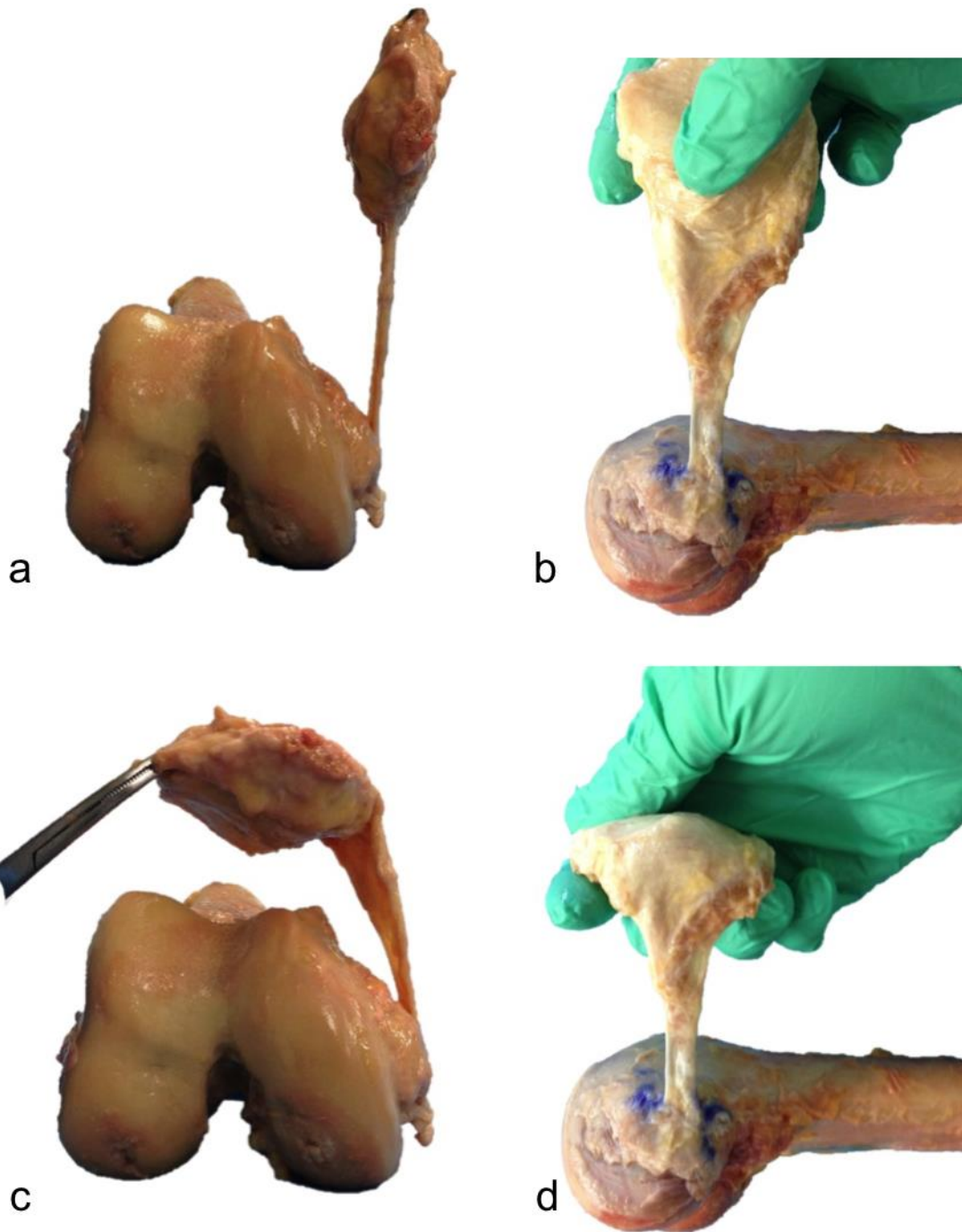


Fig. 4 – Frontal and lateral view of Non anatomical (a-b) vs. Anatomical (c-d) orientation. The MPFL was tangential to medial femoral condyle during the tensile test reproducing the physiological function in its femoral and patellar insertion.

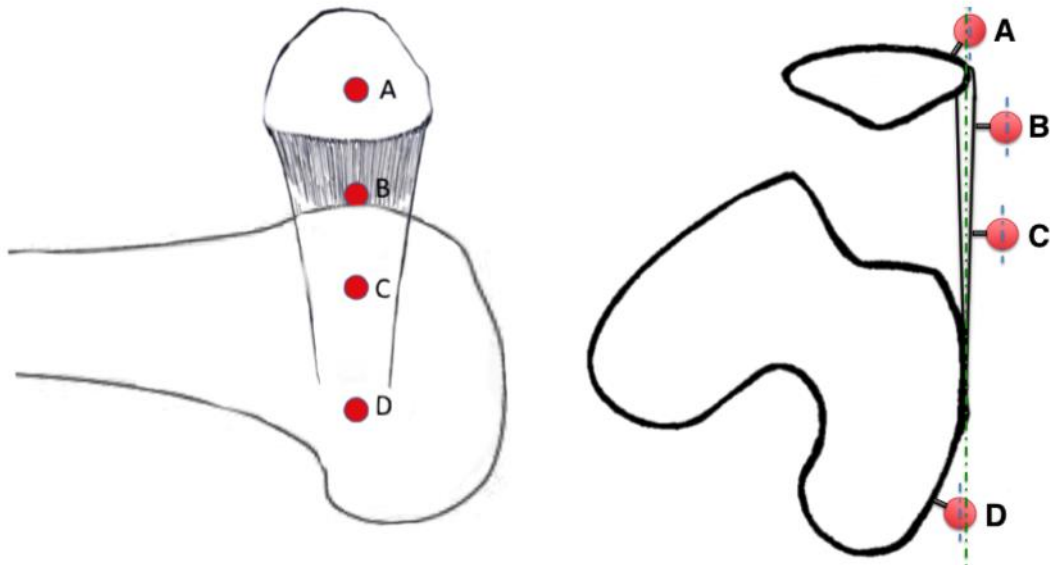


Fig. 5 – Markers setup: the four markers are positioned on different planes in space, requiring a three-dimensional reconstruction to evaluate the correct elongation.

The femur was fixed using bone cement (T-Bond hybrid resin added with portland cement (Fischer)) and four screws. It was mounted horizontally on the base frame of an Instron 5965 materials-testing machine and it was 37° externally rotated.

In this configuration, the MPFL was tangential to medial femoral condyle during the tensile test and simulated the physiological function in its femoral insertion (Burks et al., 1998).

Kim et al. demonstrated that the orientation of the specimen during tensile testing has a significant effect on stiffness and failure modes of the FMPC (Kim et al., 2014). In particular, with an anatomical orientation the angle between MPFL and patella (~90°) was preserved (Fig.4), stiffness and ultimate load of the FMPC were increased due to more uniform loading of the collagen fibers of the MPFL (Quapp et al., 1998).

For this reason, the patella was fixed in a custom clamp in anatomical orientation using bone cement, attached directly to the load cell on the moving crosshead (Fig. 3).

A preload of 1N was applied and the tissue was subjected to a 10-cycle preconditioning between 0 mm and 2 mm of extension. It was then extended at 10 mm/min to failure. From the resulting load-elongation curves, stiffness (defined as the slope of the linear region of the load–elongation

curve between 2 mm and 4 mm of extension), ultimate load, ultimate elongation and absorbed energy were determined. The mode of failure was also noted.

An optical marker-based motion capture system (Vicon Motion System) was used to evaluate the elongation of the FMPC and possible slippage or unexpected movements.

The system was composed of 4 infrared cameras and 4 markers (Fig. 5) which were positioned on the femoral condyle (marker D), on the ligament (markers C and B) and on the patella (marker A).

The use of this system is justified by the complexity of the experimental setup. In fact, compared to the case of the isolated ligament, the four markers are positioned on different planes in space and only with an accurate three-dimensional reconstruction the correct elongation can be obtained.

2.3 Statistical analysis

Data are expressed as average \pm standard deviation.

3. Results

3.1 Anatomical evaluations

The average length of the MPFL was 72.1 ± 6.6 mm (range 59 - 86 mm). The average width and thickness of MPFL for the three different locations were reported in Table 1. The average CSAs at the three locations, i.e. the patellar insertion, midsubstance, and the femoral insertion were determined (Table 1).

The results showed that, from patellar insertion to femoral one, there was an average CSA reduction of 85% caused by the variation of width and thickness. Between midsubstance and femoral insertion, a 6% reduction of the CSA was measured.

Location	Width (mm)	Thickness (mm)	CSA (mm ²)
Patellar insertion	25.9 ± 5.2	2.1 ± 0.6	55.3 ± 18.5
Midsubstance	10.6 ± 3.4	1.2 ± 0.3	11.8 ± 4.2
Femoral insertion	8.9 ± 3.4	0.9 ± 0.3	8.7 ± 4.9

Table 1 – Width, Thickness and CSA of MPFL measured in different location.

3.2 Isolated ligament uniaxial tensile tests

During the preconditioning, the samples showed the typical hysteresis of a biological soft-tissue (Fig. 6a).

During the uniaxial tensile testing of the isolated ligament, stress-strain curves representing the mechanical properties of the ligamentous tissue were obtained (Woo et al., 1999) (Fig. 6b).

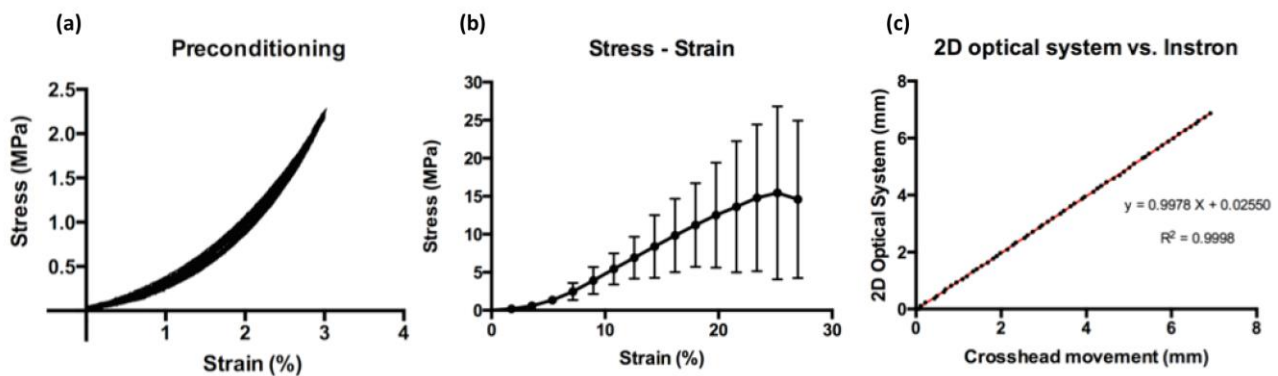


Fig. 6 – Preconditioning phase of the isolated MPFL (a); Average stress-strain curve of the isolated MPFL (b); Comparison between 2D optical system and crosshead movement (c)

The curves showed the typical stress-strain behavior of a ligament with a nonlinear toe region ranged between 0 and 5% of strain, a second linear region between 5% and 15%, and a final region where the curve reached the ultimate stress that the material can support.

Material parameters are listed in Table 2.

Material properties	Mean±SD
Ultimate stress (MPa)	16 ± 11
Ultimate strain (%)	24.3 ± 6.8
Young Modulus (MPa)	116 ± 95
Strain Energy density (MPa)	2.97 ± 1.69

Table 2 – Mechanical properties of MPFL tissues under uniaxial tension.

All the isolated ligaments failed at the mid-substance (n=12), indicating that the tensile test was appropriately set up.

The elongations, and consequently the strains, were measured both with the 2D optical system and with the crosshead movements of the tensile testing machine. The obtained results showed a very high overlap between the curves that means that no slippage or unexpected movements occurred during the tests (Fig. 6c).

3.3 FMPC uniaxial tensile tests

The parameters describing the structural properties of the femur-MPFL-patella complex include stiffness, ultimate load, ultimate elongation and absorbed energy and were obtained from the non-linear and concave upward load-elongation curves (Woo et al., 1999) (Fig.7b).

The curves showed the typical load-elongation behavior of a ligament with a nonlinear toe region ranged between 0 and 2 mm, a second linear region between 2 and 6.5 mm, and a final region where the curve reached the ultimate load that the structure can support. In the preconditioning phase, the typical hysteresis of a ligamentous tissue was showed (Fig. 7a).

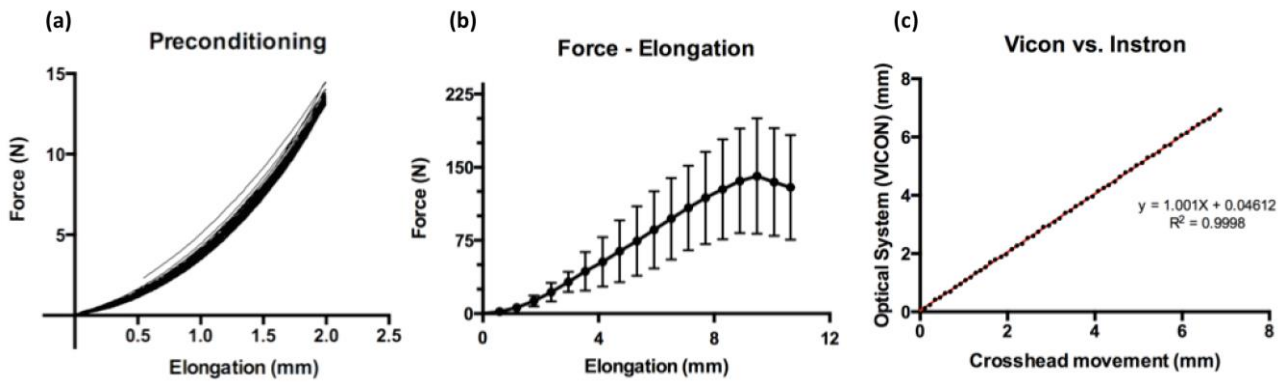


Fig. 7 – Preconditioning phase of FMPC (a); Average load-elongation curve of the FMPC (b); Comparison between 3D optical system and crosshead movement (c)

The structural parameters are listed in Table 3. Eleven FMPC failed at femoral insertion (93,3%) and only one at midsubstance (6,7%).

Structural properties	Mean±SD
Ultimate load (N)	145 ± 58
Ultimate Elongation (mm)	9.5 ± 2.9
Linear stiffness (N/mm)	42.5 ± 10.2
Absorbed Energy (N-mm)	818.8 ± 440.7

Table 3 – Structural properties of Femur-MPFL-patella complexes under uniaxial tension.

Also in this case, the elongations were measured both with the 3D optical system and with the crosshead movements of the tensile testing machine. The obtained results showed a very high overlap between the curves that means that no slippage or unexpected movements occurred during the tests (Fig. 7c).

4. Discussion

This study evaluated the tensile behaviour of the medial patello-femoral ligament and proposed a distinction between mechanical properties of ligament substance and structural ones of FMPC. This difference is significant because the MPFL is characterized by a variable cross sectional area

that influence the distribution of the load.

The isolated ligament uniaxial tensile tests showed the typical stress-strain curve of a ligament. In this curve three different regions can be identified (Woo et al., 1999). In the nonlinear toe region with a low initial stiffness, the collagen fibers were easily extended and small initial forces produced large elongations. The second region was linear and characterized by higher stiffness (the slope of the curve) which derives from the elongation of the fibers. Finally, in the last region the curve reached the ultimate load that the structure can withstand. Exceeded that load, the failure of the ligament occurred.

All the isolated ligaments failed at the mid-substance ($n = 12$), indicating that the tensile test was appropriately set up.

However, until the 90's, limited information were known about the function of MPFL, and many authors discussed about its existence or considered it as an inconsistent structure of the knee (Reider et al., 1981). In literature, no studies related to the mechanical properties of MPFL were found whereas different studies were performed to evaluate biomechanical properties of other ligaments. In particular, Quapp et al. performed the material characterization of Medial Collateral Ligament (MCL) (Quapp et al., 1998). They found the typical stress-strain curves of a ligament and the average tensile strength, ultimate strain, and tangent modulus for the longitudinal specimens were 38.6 ± 4.8 MPa, 17.1 ± 1.5 %, and 332.2 ± 58.3 MPa, respectively. These results were comparable with those obtained in our study and represented another piece of evidence for the existence and consistence of the MPFL as a ligament.

The results obtained with the FMPC uniaxial tensile tests showed that the ultimate load of the complex was 145 ± 58 N. This value was acceptable for specimens with a mean age of 75 years, because the effect of the age (Woo et al., 1991) reduced the mechanical and structural properties of the MPFL that, at a visual inspection, resulted transparent and thin. These results are supported

by Burks et al. (Burks et al., 1998) who noted a peak of 209 N at 25 mm of displacement for failure of the MPFL during lateral patellar dislocation. Similar result (208 N) was found (Mountney et al., 2005): in this study, the femur position is the same as in our study, the patella instead, was mounted in a V-shaped clamp attached to the load cell. We preferred to preserve the anatomical orientation the angle between MPFL and patella (90°) (Fig. 4).

Completely different testing parameters were also chosen: specimens were preconditioned by ten cycles from 0 N to 30 N and then extended at 200 mm/min until failure. In our study, the precondition phase was position controlled, and the load was around 15 N; the elongation speed was 20 times smaller. Considering that the viscoelastic behavior of ligaments, this last discrepancy can explain the difference in ultimate load, also because the donor age is similar (71.6 ± 16.6). Arendt (2009) (Arendt et al., 2007) indicated that the ultimate load was 145.6 N no other information is provided.

Specimens failures, eleven FMPC failed at femoral insertion and only one at midsubstance, confirm that the anatomical orientation of the MPFL with respect to the patella affects the results of tensile testing. In fact, with an anatomical orientation, the angle between MPFL and patella was preserved, the fibers were uniformly loaded, and failures occurred at the femoral insertion where the cross-sectional area is smallest. In the anatomical orientation, femoral insertion became the weakest link, which explains why it failed consistently at the femoral attachment.

Comparing the experimental data obtained from the isolated ligament and FMPC, different correlations were found. In particular, assuming a uniaxial state of stress during the FMPC test (this hypothesis is reasonable thanks to the experimental setup), the smallest section of ligament, proximal to the femoral insertion, experienced an ultimate stress of 16.7 ± 4.0 MPa, very close to the ultimate stress calculated for the isolated ligament.

Although the precautions taken into the entire experimental design, a limitation of this study can

be related to the method used to evaluate the cross sectional area. The use of digital caliper and micrometer introduced an error related to the precision and accuracy of the operator. A possible solution would be the use of a laser micrometer system or a 3D scanner in order to detect the real shape of the structure and, subsequently, the CSA with high precision. However, despite this limitation, the obtained values result to be consistent with the other ones present in literature (Burks et al., 1998; Mountney et al., 2005; Arendt et al., 2007; Arendt, 2009), and show consistency between material and structural properties (e.g. the ultimate load).

The material and structural properties of MPFL obtained in this study can help in understanding MPFL contribution as stabilizer and for the selection of repair of this ligament. Two different strategies can be followed to reach a complete and long-term functional repair of the damaged tissue: reconstructive and regenerative approaches. Different points of view regarding reconstructive methods have been presented, focusing on the graft choice and tension, knee flexion angle and fixation methods. Many of these methods have been compared, studying the stiffness of the reconstructed ligament and the elongation of the graft after cyclic tests; results are still controversial (Mountney et al., 2005; Lenschow et al., 2013) but a complete characterization on material and structural properties can help in the choice of the best graft and surgical technique. The regenerative approach represents a new strategy based on Tissue Engineering (TE) that aims at fabricating an immunological tolerant tissue substitute to permanently restore the functionality of the damaged one, without the need for supplementary therapies (Sachlos and Czernuszka, 2003). Nevertheless, important challenges must be solved to obtain complete ligament repair that will lead to a clinically effective and commercially successful application (Rodrigues et al., 2013): it is well known that the fate of cells seeded onto a scaffold is extremely dependent on the mechanical properties of this substrate (Engler et al., 2006). Indeed also for the regenerative approach, the complete mechanical characterization of the tissue is a necessary

starting point for the design of a scaffold for the regeneration of the MPFL.

Conflict of interest statement

All authors confirm they have no financial or other conflict of interest relevant to this study

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