Structural-mechanical characterization of human ligaments using a custom-made tensile test chamber combined with the Skyscan1176 system

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Aims

Anterior cruciate ligament (ACL) injuries are one of the major and one of the most frequent clinical problems in orthopedics[1]. Connecting femur and tibia, the ACL decreases the joint degrees of freedom and maintaines joint stability under load conditions. Thus, ACL plays a fundamental role in the stability and functionality of the knee. When injured, the common approach lies on its surgical reconstruction by using several types of graft[2]. However, surgical or implant reconstruction techniques still show unsatisfactory long-term success. Therefore, the knowledge of the biomechanical, structural and morphological properties of native tissues is fundamental in order to reduce the need of surgical revision and the probability of early development of other pathologies, such as osteoarthritis. This better understanding of the properties of ACL could help to optimize the choice and the development of new grafts and scaffolds.

Dense connective fibrous tissues, such as ACL, typically exhibit non-linear stress-strain characteristics related to fibers crimp, orientation and tension, which intrinsically define its functional properties. The behavior of ACL bundles in relation of its fibrous structure has already been studied under load, but considering only the outer surface and not the entire 3D structure[3]. Aim of this study was to design and test a dedicated setup able to acquire the volumetric fibrous microstructure of the ACL samples, under progressive increasing mechanical strain.

Method

The low intrisic X-rays contrast of soft tissue leads to a difficult identification of ACL fibrous structure. First, we thus tested which contrast enhancement agent could be optimal for this purpose. We carried out different tests using different contrast stain formulation, concentrations and protocols (we used solutions of iodine or PTA).

Once we had defined the optimal contrast protocol for fiber visualization / reconstruction using micro-CT, we proceeded to design the load cell and combine it with the 1176 system. The overall dimensions (Figure 1), the materials, the design and realization of the clamps, etc. were considered and analyzed.

The design of the load equipment was performed using PTC Creo 4.0 software, a CAD solution for 3D digital projects. The structural non-commercial components are made of Ergal, an alloy of aluminum and zinc. This material, when compared to stainless steel, is characterized by a lower density (2.81 g/cm³) and a good mechanical resistance and has a lower x-ray attenuation.

Figure 1: Overall involved dimensions' considerations.

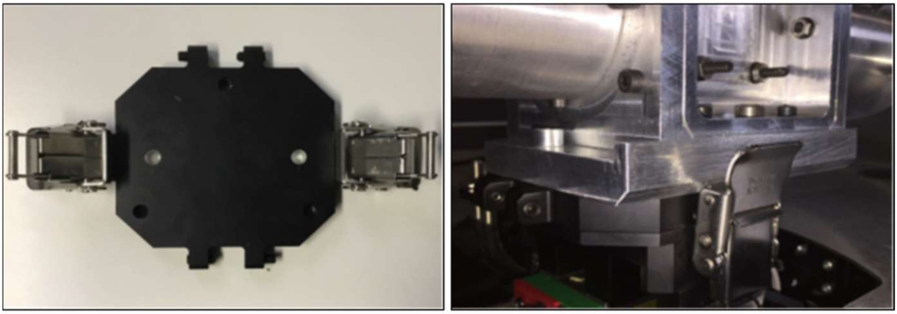

Figure 2: Clamping system.

To attach the structure to the micro-CT system we used the basement for the carbon fiber animal bed of the 1176 (Figure 2).

The final equipment (Figure 3) is a device consisting in an actuation and support side and in a measuring side. The former consists in a cylindrical tube and a cube-shaped support fixed to the scanner basement that contains a linear guide driven by a manually operated threaded rod (0.75 mm pitch) to impose axial elongation to the ligament specimen. The measuring side consists in three coaxial cylindrical tubes that support and surround two clamps that grasp the ligament specimen for the tensile testing and a load cell to measure the axial load on the specimen. In particular, the two clamps are connected to the linear guide and to the aluminum tube respectively. Moreover, the central tube has a calibrated 0.5 mm thickness to reduce x-ray attenuation and resist up to a 1000 N compression force.

The axial load cell inside the machine is the model 8431-6001 (resolution 1N, full scale 1000N) supplied by Burster Italia s.r.l. It is a product with reduced dimensions and suitable for measuring both tensile and compressive loads. To acquire the signal of the load cell we used the analogue input terminal EL3356-0010 and the EK1100 coupler supplied by Beckhoff Automation s.r.l.

Afterwards, we developed a graphical interface to display on the computer the signal sent by the load cell using TwinCAT 3 (Beckhoff Automation s.r.l.) and Microsoft Visual Studio softwares. We created a Simulink project (MathWork Inc.), set its acquisition on Visual Studio and consequently connected the load cell to the interface: the EK1100 coupler and the EL3356-0010 terminal.

The device was then calibrated to adjust the instrumentation and allow subsequent tensile tests on human ligaments.

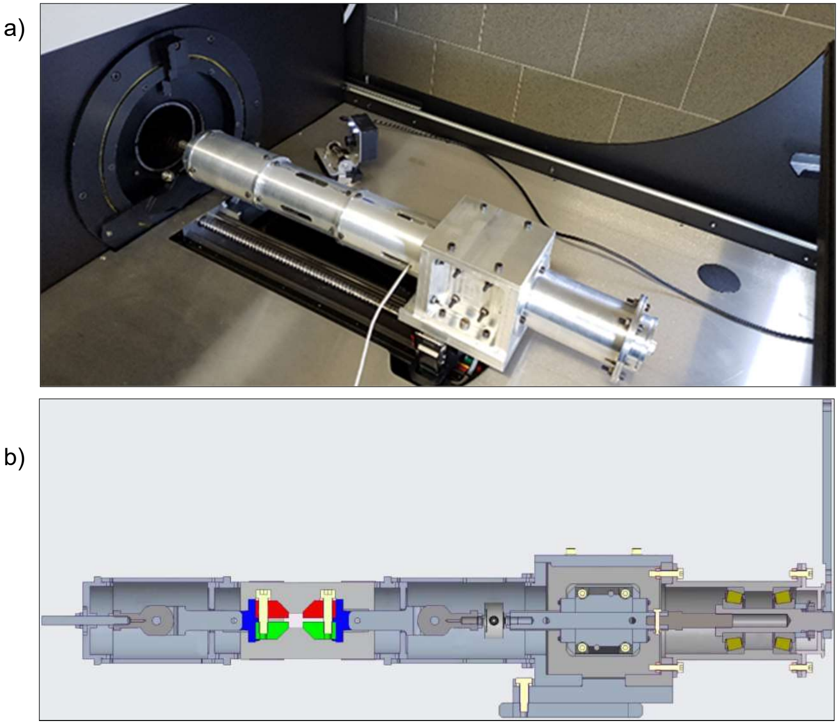

Figure 3: a) Custom-made ligament tensile system and b) its schematic section.

After the design and assembly phases of the custom-made tensile test chamber, we carried out experimental tests to study the behavior of human ACL samples subjected to tension, and to visualize the fibers' reaction.

We collected and froze fresh ACL samples from patients that underwent surgery for total knee replacement. The study was approved by the local ethics committee.

In detail, after defreezing, ACL samples were stained in a 2% PTA solution in H_2O overnight to highlight the collagen fibers. Then, ACL was clamped and its elongation was manually imposed by acting on the threaded rod and measuring the screw angle, while the reaction force was measured by the load cell. First, ACL was subjected to cycles of preconditioning to reduce the effects of freezing according to specific procedures. Then, it was subjected to subsequent percentages of deformation (1, 2, 4, 6, 8 and 10%) to detect the toe- and linear region of the stress-strain curve. After each elongation of the ACL, the tension was maintained for 12 minutes to exhaust stress-relaxation. In order to obtain the visualization of the ACL fibers, the sample was scanned using the 1176 micro-CT system at each specific elongation value. A source voltage of 50 kV and a source current of 500 uA was applied for the acquisitions. The nominal resolution used for the images was set at 9 µm (pixel size). The images (2672x4000 pixels) were then reconstructed with NRecon program (version 1.7.1.6, Bruker) to obtain the micro-CT sections in bmp format (4000x4000 pixels, maintaining the relative pixel size). In addition to the specific alignment, slight beam hardening and reduction of ring artifact were used as correction factors in the reconstruction process.

Results

In this paper, we want to show the feasibility of the study with some preliminary results obtained by the analysis of a human ACL sample.

We applied a preload of 20 cycles (6 s each), oscillating between 22 N and 110 N, followed by the application of a load of 22 N for 720 s. The sample length was measured maintaining a constant load of 22 N: L_0 = 13.75 mm. Afterwards, axial strain of 1%, 2%, 4%, 6% and 8% were applied with respect to L_0. At each elongation of the ACL, a micro-CT dataset was acquired with a 14 minutes scanning. The progression of the reaction exerted by the ligament during the test and the force-elongation curve are shown in Figure 4.

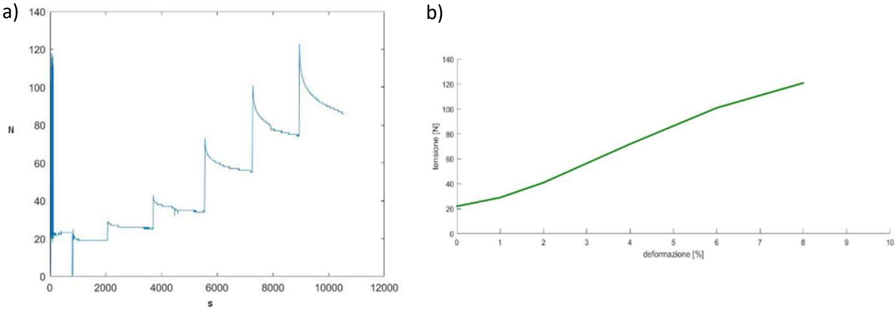

Figure 4: a) Force (N) exerted by the human ligament during the trial as a function of time (s); b) the obtained overall interpolated force-elongation curve.

This preliminary test revealed sample strain-stress behaviour in fair agreement with the literature[4], even if some of the test conditions may have partially altered mechanical behaviour. The micro-CT images obtained at 0% of deformation and at 8% of deformation are shown in Figure 5.

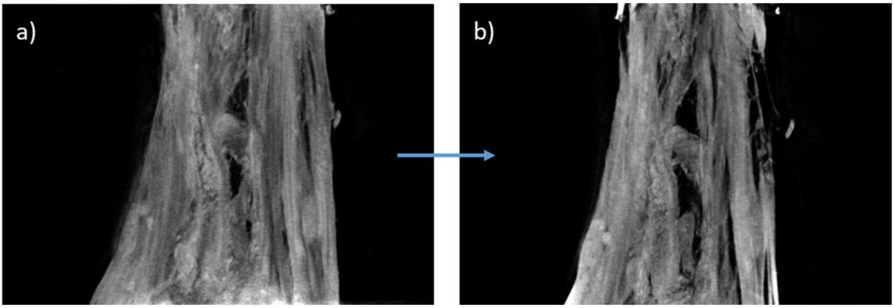

Figure 5: Coronal micro-CT sections of the ACL at 0% (a) and 8% of elongation (b).

Conclusion

In this study, using a specific micro-CT procedure with a nominal resolution of 9 µm, we were able to detect the ACL hierarchical structure at the level of the fibre bundles (Figure 6). Moreover, thanks to the designed experiment, it was possible to visualize the microstructure of ACL bundles under progressive longitudinal elongation and aquire images at different % of strain over time. At present, we are working on image analysis and processing in order to perform a 3D morphometric analysis on the fibre bundles' changes over time in relation to the quantified mechanical behavior. In the future, developing an evolution of the proposed device, this approach can be also exploited to compare native ligament and natural/artificial grafts, even under torsional conditions.

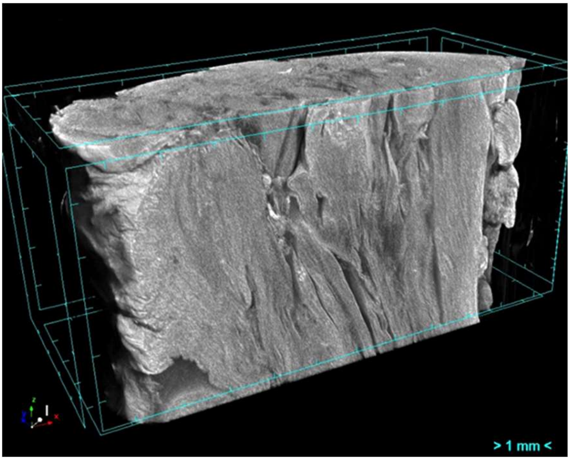

Figure 6: 3D CTVox model of the fibre bundles of the ACL.

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