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Mechanical behavior of rat tendons under cyclic tensile loadings

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OBJECTIVE

A tendon is a multiscale microstructure made of helical collagen molecules, microfibrils, fibrils, fibers and fascicles (Fig. 1a). It results a complex mechanical behavior (which is also coupled with bio-chemical phenomena). Tendinitis is a common disease of the tendon which if untreated can result in serious long-term complications. It is generally treated using nonsteroidal anti-inflammatory drugs (NSAID) which give good results against pain. An alternative to drugs is Low Level Laser Therapy (LLLT). The aim of this study is to compare the effect of LLLT and NSAID treatment on the mechanical properties in the case of acute inflammation of tendon. In a first step, indicators of the mechanical performance of tendons are proposed. In a second step the link with the microstructure is discussed to find more pertinent indicators of the general mechanical properties of tendons.

INTRODUCTION

To characterize the mechanical behavior of the tendon, the current literature refers primarily to the strength and elongation at rupture and to the stiffness measured in the linear region of the tensile curve (Sahin et al., 2012). These mechanical properties correspond to the behavior under monotonic loading which doesn't reflect adequately the in vivo conditions. In this study, cyclic loading have been performed on rats tendons after different treatments. Numerous mechanical properties were extracted from each test. There modifications were evaluated and linked to the acute inflammatory process and treatments. The correlations between mechanical properties and biochemical indicators have been analyzed (Marcos et al., 2013).

APPROACH

The experimental protocol was conducted as described in a previous paper (Marcos et al., 2013).

Samples preparation

Tendinitis was induced in a group of male Wistar rats using collagenase, an enzyme that degrades collagen and induces an acute inflammatory process. The same procedure was used for a control group (CTL) without collagenase. A first group (DIC) was treated using diclofenac sodium (Voltaren injectable® – Novartis 2.5 mg/kg). Two other groups (L1J and L3J) were treated by laser irradiation with doses of 1 J and 3 J respectively. The last group (TEN) was not subjected to any treatment of tendinitis. The animals of the DIC, L1J and L3J groups were treated in the same conditions for 6 days. On day 7, animals were sacrificed with halothane. Achilles tendons were dissected and immediately used for biomechanical analysis.

Biomechanical Analysis

Musculotendinous junction (MTJ) was fixed in the lower grip as osteotendinous junction (OTJ) was fixed in the upper one using a universal tensile test machine (zL2.5 Zwick-Roell – Germany). The distance L_0 between the grips (Fig. 1b) was measured before each test, its mean value was approximately 2 mm. Displacement u was controlled following loading and unloading cycles at a constant velocity of 1 mm/min. At each cycle, maximal displacement applied to tendon was increased of 10% of L_0 and tensile force F was released until 0.1 N. The sequence was repeated until rupture (Fig. 2).

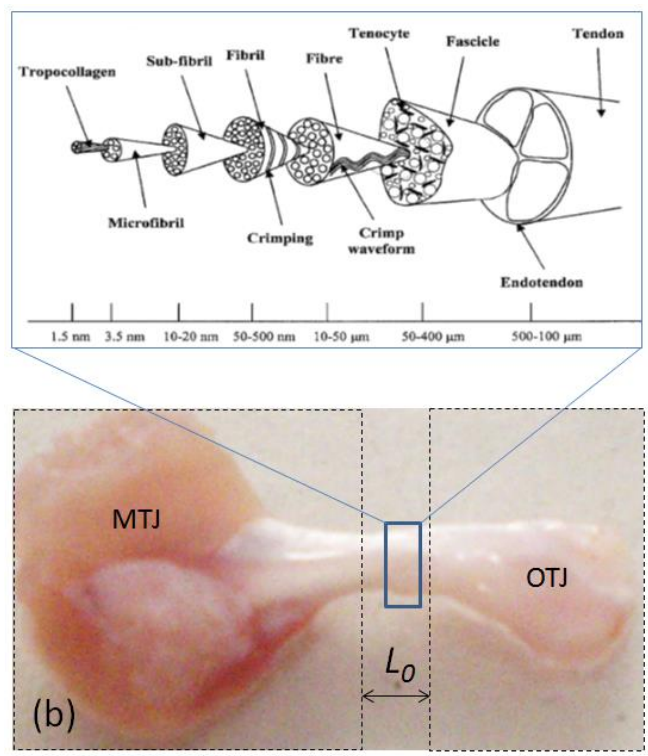

Figure 1: (a) Multiscale microstructure from tendon (from Screen *et al.*, 2005); (b) Rat Achilles tendon.

Several mechanical characteristics were extracted from the force – displacement curves obtained from cyclic loadings of each tendon. The consideration of numerous characteristics enables description of tendon mechanical behavior without to prejudge the choice of a particular set of parameters. Three stiffnesses were measured at each loading – unloading cycle (indexed i) as illustrated in Fig. 2: secant stiffness k_s as the load / displacement ratio; tangent stiffness k_t as the local slope of the envelope of the loading curve; unloading stiffness k_u as the slope of the curve immediately after a maximum point M_i (Fi , u_i):

$$k_s^{\,i} = \frac{F_i}{u_i}$$

$$k_t^{\,i} = \frac{F_i - F_{i-1}}{u_i - u_{i-1}} \quad \text{with } i = \{1 \ldots n\} \text{ and } (F_0 = 0,\ u_0 = 0)$$

$$k_u^{\,i} = \left.\frac{\partial F}{\partial u}\right|_{dF<0} \left(M_i^{+}\right)$$

The stretch λ was defined as the ratio L / L_0 where L and L_0 are respectively the current and initial distances between the two grips of the loading device ($L = L_0 + u$). The maximum value of the force F_{max} and the corresponding value of the stretch λ_{Fmax} characterize the mechanical strength in term of load and elongation. The maximum of each stiffness was measured: $k_{s\ max}$, $k_{t\ max}$ and $k_{u\ max}$, as well as the corresponding force and stretch values: ($F_{ks\ max}$, $\lambda_{ks\ max}$), ($F_{kt\ max}$, $\lambda_{kt\ max}$) and ($F_{ku\ max}$, $\lambda_{ku\ max}$) respectively. Finally 11 measured values were considered for each tested tendon.

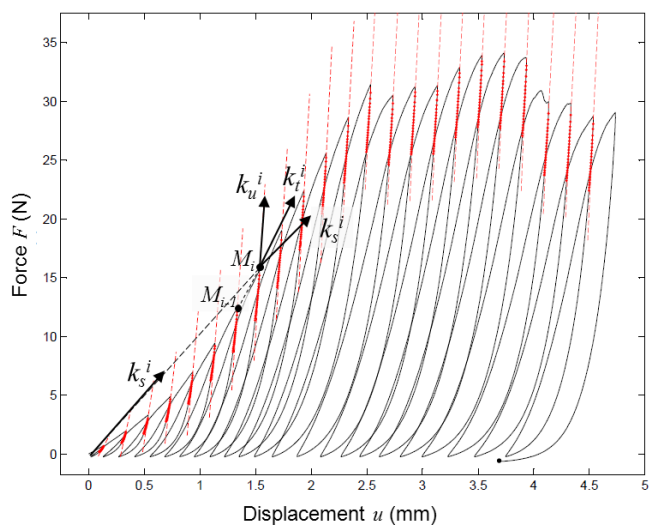

Figure 2: Definition of the 3 different stiffnesses at each cycle.

RESULTS AND DISCUSSION

Representative load – displacement curves are shown in Fig. 3 for the DIC and L1J groups as examples. Envelope curves exhibit the typical response obtained for tendon under monotonic loading: *i.e.* a "toe" region, followed by a "linear" part up to a maximum (λ_{Fmax}, F_{max}) beyond which the force decreases. This evolution is commonly explained by the helical shape of the collagen molecules (Grytz et al., 2009) and by the well-known phenomena of gradual recruitment of collagen fibers. Large differences of load levels were also observed. Because of the shape of the load – displacement envelope curves, the evolution of stiffnesses k_s and k_t with displacement u (Fig. 3) exhibited an increasing phase, a maximum and a final decreasing phase where k_t reached negative values. If the initially increasing evolution of k_t was linked to the reorientation of the fibers, the suddenly decreasing evolution of k_t could be explained by a gradual fracture of the fibers that could be described using a damage variable (Weisbecker et al., 2012). The evolution of the unloading stiffness k_u with displacement was different but could also be partially explained by damage: it increased linearly up to saturation close to the maximum load. One can note that the maximum value of the secant stiffness k_s was reached when the secant was tangent to the envelope curve, corresponding to the maximum global stiffness of the tendon. Tangent stiffness k_t and unloading stiffness k_u describe the response of the tendon under an increasing or a decreasing load, respectively. The tangent stiffness k_t reveals the ability of the tendon to resist to a force increment at a given force level. The unloading stiffness values were much higher than the values of secant and tangent stiffnesses. This difference could be related with the viscoelastic behavior that was not assessed in this study.

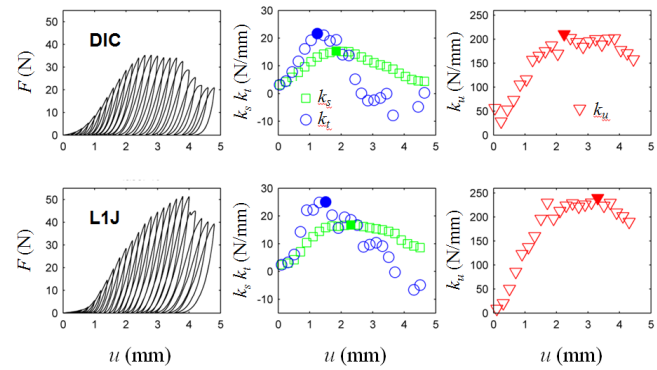

Figure 3: Representative F-u curve, k_s ,k_t and k_u evolution for DIC and L1J groups.

CONCLUSION AND FUTURE WORK

In the framework of this study, the definition of different stiffnesses has enabled to describe the evolution of the mechanical properties of the tendons under cyclic loading. In a future work, models of the literature could be adapted and identified using these results in order to assess more accurate information on the behavior like viscosity and damage.

KEYWORDS

Tendon, LLLT, stiffness, tensile behavior

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