Structure–mechanical properties relationship of natural tendons and ligaments

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The mechanical characterization of rabbit Achilles' tendon and anterior cruciate ligament is presented. Both static and dynamic mechanical tests have been performed on fresh explanted tissues. Experimental results are presented and discussed in terms of a relationship between structural architecture and mechanical properties. The differences in collagen fibre configuration and composition between tendon and ligament influence the tensile and viscoelastic properties. In particular, tendons and ligaments showed a gradual transition from a rubber-like to a glassy-like behaviour during the loading process due to the traightening of collagen fibres.

1. Introduction

The main mechanical function of tendons and ligaments is to transmit loads between the elements that they connect. The tendons link together muscles and bones ensuring mechanical continuity, transmitting tensile load from the muscle to the bone and producing, therefore, joint motion. The ligaments connect the articular extremities of the bones reducing the degrees of freedom of the articulation by constraining non-physiological movements. Tendons and ligaments accomplish their various mechanical tasks thanks to a synergistic cooperation among the constituents of their own structure.

Tendons and ligaments are dense connective tissues constituted by a protein phase (collagen and elastin) and a polysaccharide phase (proteoglycans). The overall mechanical properties are determined, apart from the relative amount of the two phases, by geometrical factors, conformation and orientation of the single constituent [1]. The structural architecture of the tissue plays a very important role in the determination of mechanical properties. In fact, tissues formed by the same elements show completely different behaviours. The skin and tendons are a practical example that confirms this assertion [2]. The collagen fibres in the skin are randomly dispersed in the polysaccharide substance without any preferential orientation, and the mechanical properties are quasi-isotropic. In the tendons, in contrast, the collagen fibres are arranged along the axial direction rendering the material anisotropic and thus showing higher stiffness in the fibres direction. These different properties respond to the different requirements that the two materials have to satisfy: the skin has to resist isotropically to multiaxial external forces, the tendons are predomi nantly subject to axial tensile stress.

The microarchitecture of the cruciate ligaments has often been linked to the hierarchical arrangement described for tendons, in which collagen fibrils are grouped into fibres that, in turn, make up subfascicular units that are bound together to form a fasciculus [3, 4] that in turn is enclosed in sheaths of loose connective tissue. The ACL (anterior cruciate ligament) has a complex structure which can be separated into two major bundles: an anteromedial bundle and posterolateral bundle. The bundles are oriented in different directions, causing the ACL to twist medially and to resist multiaxial stresses. In tendons the collagen fibres have a strict waveform parallel arrangement to resist unidirectional tensile load.

The mechanical properties and configurations of collagen give tendons and ligaments their characteristic strength, flexibility and an ideal physical adaptation for tensile force tansmission. The collagen content in tendons and ligaments is different: in tendons the collagen concentration is about 87%, while in ligaments it reaches about 80% [5].

The mechanical properties of tendons and ligaments depend not only on the properties and arrangement of their constituents but also on the proportion of various components. Elastin, scarcely present in tendons and extremity ligaments, probably enables the natural structures to recover imposed deformations. Elastin, present in ligaments only in small amounts (about 5%), forms networks interdigitated among ligament collagen fibril fascicle contributing to the organization and function of the ligament matrix.

Water contributes about 60% or more of the wet weight of most ligaments and tendons, and in association with proteoglycans provides the lubrications and spacing that are crucial to the gliding function at

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the fibre–matrix interface. Water and proteglycans confer the required viscoelastic properties to the tissue structure [6].

The mechanical properties of tendons and ligaments depend also on the presence of fibrocartilage and their insertions. Fibrocartilage might afford a gradual change in elastic moduli at insertion sites due to the variation in mineralization. The change of elastic modulus at an insertion site decreases the chance of failure, i.e. enhances the ability of the insertion to dissipate deformation energy [6].

The aim of this work was to look at the relationship between the structural microarchitecture and mechanical properties of tendons and ligaments, with particular emphasis on the role of conformation and orientation of the collagen fibres in determining the mechanical behaviour of these tissues. Both static and dynamic mechanical tests have been performed for this purpose, and the results have been interpreted on the basis of the composite architecture.

2. Materials and methods

2.1. Rabbit anterior cruciate ligament and Achilles' tendon

Fresh anterior cruciate ligaments and Achilles' tendons were explanted from ten New Zealand White rabbits. For ligament testing the skin, muscle capsule and all the other ligaments were excised to isolate the ACL. The upper half of the femur and the lower half of the tibia were sawn off. The femur and tibia were fixed in specially designed clamps by means of 3 mm diameter pins. One pin was drilled in the latter femoral epicondyle as near as possible to the axis of motion to permit free rotation of the femur. The other hole was drilled transversely in the tibia. The whole system was then mounted on a testing machine at about 70° of external rotation with respect to the femur (Fig. 1). This kind of assembly gave knee adjustment sufficient to obtain the best alignment of the ligament with the tensile load directions.

The explanted Achilles' tendons were clamped using special gummed grips in order to avoid slippage during the test. All experiments were performed within 1 h after sacrificing the rabbit. During the preparation of the specimen, the ligaments and tendons were covered by a wet paper towel to prevent dehydration.

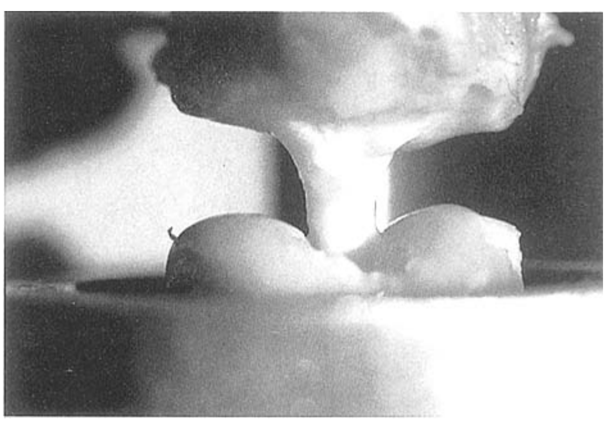

Figure 1 Anterior cruciate ligament–bone system.

2.2. Tensile properties

Uniaxial load–strain experiments were made by using a standard Instron testing machine Mod. 4204 at a temperature of 37 °C in physiological solution. A strain rate of 0.1 min^{-1} was used for all tests.

2.3. Dynamic-mechanical properties

Viscoelastic parameters (E' and E'') were measured by dynamic mechanical tests using a Dynastat (IMASS Inc.) apparatus operating in tension. A Dynatherm system was used to give accurate temperature control at 37 °C.

The static load was varied from 10 N to 90 N and all tests were performed with a 5 N dynamic load. The frequency was scanned from 0.4 to 40 Hz.

3. Viscoelastic analysis

The term 'viscoelastic' refers to materials which show elasticity and at the same time viscous behaviour. The elastic response is attributed to the instantaneous and reversible deformation under external load, while slow relaxation due to configurational rearrangement of the material characterizes the viscous response. The ratio between deformation rate and molecular configuration relaxation velocity determines the mechanical response of the material [7]. When the deformation rate is well beyond the characteristic velocity of the relaxation phenomena, elastic behaviour is attained, while at the other extreme (i.e. when the deformation is slower than relaxation) viscous behaviour results. Viscoelastic response is attained when the two characteristic velocities are of the same order of magnitude. Virtually all materials are viscoelastic in nature, but this behaviour is much more marked for macromolecular materials in which poor molecular mobility renders the characteristic relaxation velocity of the same order of magnitude as the commonly used deformation rate [8].

Viscoelastic analysis is usually carried out by imposing a sinusoidal excitation function and monitoring the resultant response [9]. If a sinusoidal force is imposed on the sample, the deformation response of the material is composed of two components: elastic (in phase with excitation) and viscous (out of phase). The total response will lag behind the excitation by a phase angle ϕ (Fig. 2). The phase lag results from the time necessary for molecular rearrangements and is associated with the relaxation phenomena. The stress

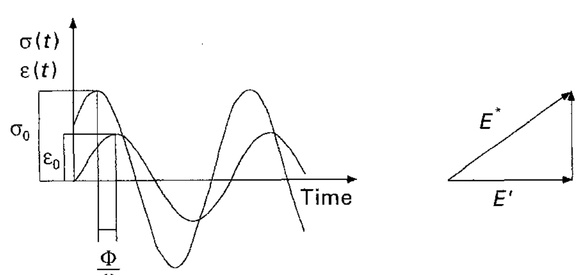

Figure 2 Representation of the parameters used for dynamic mechanical measurements.

σ and the strain ε can be expressed as follows:

$$\sigma = \sigma_0 \sin(\omega t + \phi)$$
$$\varepsilon = \varepsilon_0 \sin \omega t$$

where ω is the angular frequency. Applying simple trigonometric rules, we have:

$$\sigma = \sigma_0 \sin \omega t \cos \phi + \sigma_0 \cos \omega t \sin \phi \qquad (1)$$

where $(\sigma_0 \cos \phi)$ and $(\sigma_0 \sin \phi)$ are the in-phase and out-of-phase components, respectively. The in-phase modulus (elastic) and out-of-phase modulus (viscous) can be obtained dividing relation (1) by the strain:

$$E' = (\sigma_0/\varepsilon_0)\cos\phi \qquad E'' = (\sigma_0/\varepsilon_0)\sin\phi$$

The vector composition of E' and E'' in the rotating vector plan lead to the complex modulus E_0^*. The elastic part of the complex modulus is also called the storage modulus, being correlated with the part of energy that is stored in the material; the viscous part E'' is also called the loss modulus, being correlated with the dissipated energy.

4. Results

Static load–strain curves of fresh rabbit Achilles' tendon and ACL are reported in Fig. 3. These curves, according to many others relative to collageneous tissues and reported in the literature [10, 11], show an upward concavity with an initial low modulus region (toe region) followed by a progressive increase in modulus up to a "linear" load–strain characteristic. Linear behaviour held up to failure of the specimen, which happened at around 40 MPA for tendons and 30 MPA for ligaments. By comparison of the two curves it can be seen that ligament presents a wider toe region and consequently a lower initial elastic modulus. These behaviours can be easily explained in terms of the differences in collagen fibre composition and configuration in tendons and ligaments. Moreover, the latter contains a higher proportion of elastin and this results in greater elongation before fracture [12].

Natural tendons and ligaments are viscoelastic materials [13, 14]. This term implies that the mechanical behaviour cannot be described in terms of purely elastic moduli alone but must also include a "viscous" or time-dependent contribution. As outlined earlier, the dynamic behaviour can be characterized by just two parameters, typically the storage and loss moduli. These properties are functions only of frequency and temperature and they must be determined experimentally.

However, the theory of linear viscoelasticity allows only a linear dependence of stress upon strain. The stress–strain curves of natural ligaments and tendons are non-linear. This fact represents the most obvious departure of the viscoelasticity of natural ligaments and tendons from the simple theory outlined above. For the purposes of correct viscoelastic characterization several dynamic mechanical tests were performed in a load control mode at an increasing level of static stress (ranging from 10 to 100 N). A check of "local" linearity was done at all levels of static stress and for this purpose the amplitude of the dynamic stress was chosen in the range of 5 N to 10 N. Figs 4 and 5 report the dependence of storage modulus upon frequency for ACL and Achilles' tendons, respectively. An increase of the storage modulus with frequency was observed for all levels of static load. The slope of the curves slightly decreases with increasing static load. The storage modulus as a function of static load is

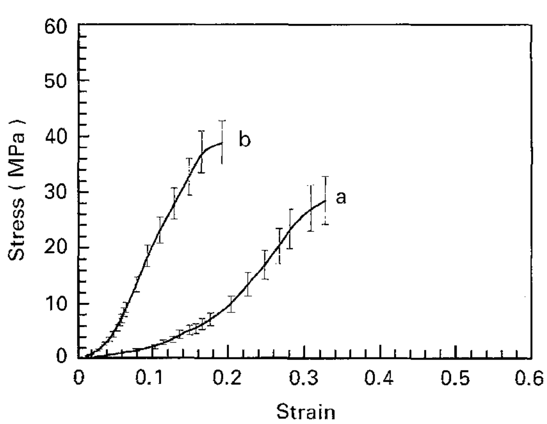

Figure 3 Stress–strain curves for rabbit ACL (a) and Achilles' tendon (b).

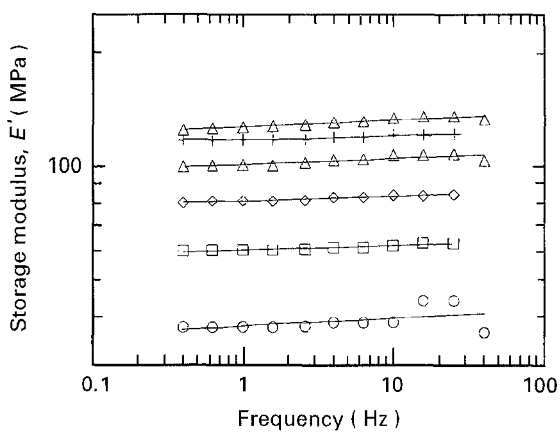

Figure 4 Storage modulus versus frequency for ACL at dynamic load 5 N and static load: —○— 15 N; —□— 10 N; —◇— 20 N; —△— 40 N; —+— 60 N; —▽— 90 N.

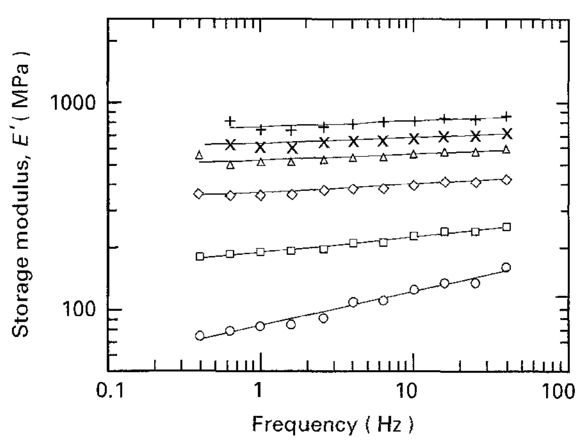

Figure 5 Storage modulus versus frequency for Achilles' tendon at dynamic load 5 N and static load: —○— 5 N; —□— 10 N; —◇— 20 N; —△— 30 N; —×— 40 N; —+— 60 N.

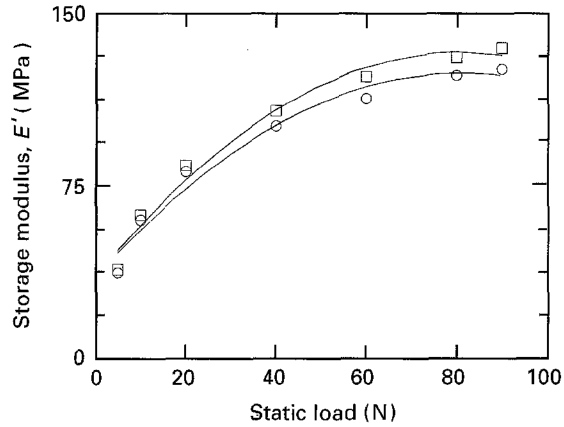

Figure 6 Storage modulus versus static load for ACL at dynamic load 5 N: —○— 1 Hz; —□— 10 Hz.

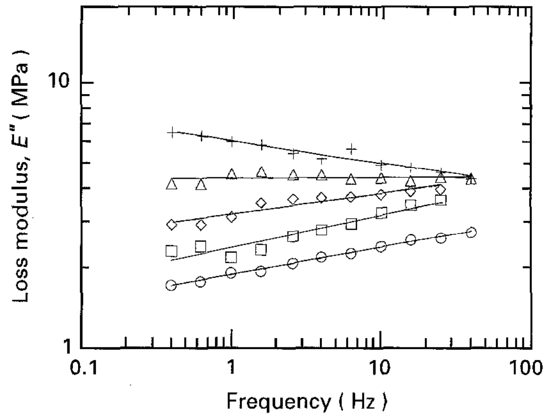

Figure 8 Loss modulus versus frequency for ACL at dynamic load 5 N: —○— 5 N; —□— 10 N; —◇— 20 N; —△— 40 N; —+— 90 N.

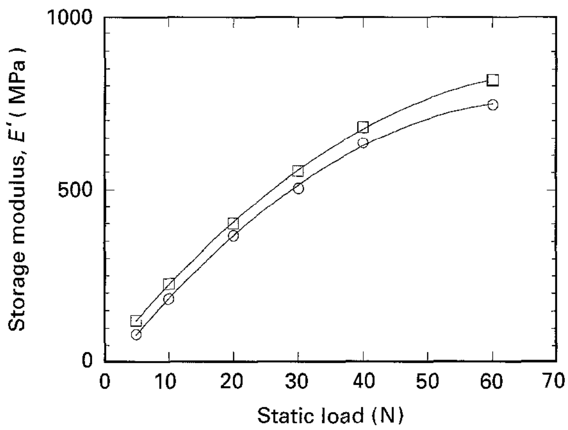

Figure 7 Storage modulus versus static load for Achilles' tendon at dynamic load 5 N: —○— 1 Hz; —□— 10 Hz.

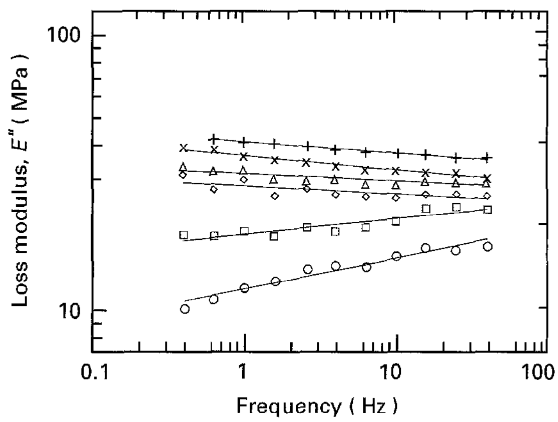

Figure 9 Loss modulus versus frequency for Achilles' tendon at dynamic load 5 N: —○— 5 N; —□— 10 N; —◇— 20 N; —△— 30 N; —×— 40 N; —+— 60 N.

reported in Figs 6 and 7 for ACL and Achilles' tendon, respectively. These diagrams are derived from Figs 4 and 5, and show clearly the dependence of elastic modulus upon static load at two different frequencies, namely 1 and 10 Hz. The curves reflect the increase of the modulus at lower static load and the levelling off at higher stresses, as expected from the static stress–strain curves reported previously. The curves, as for the static case, show clearly "toe" (low modulus) and "linear" (constant modulus) regions.

Figs 8 and 9 report the dependency of the loss modulus, E'', upon frequency at different levels of static load. At low static load, an increase of loss modulus with frequency was observed, while the opposite trend was evident at higher static load. These curves show very clearly the change in behaviour of the structures during loading. The increase of loss modulus with frequency at low static load is typical of rubbery materials, while the decrease of loss modulus with frequency at higher static load is characteristic of glassy materials [7]. Figs 8 and 9 show that tendons and ligaments are subject to a gradual transition from rubber-like to glassy-like behaviour during the loading process. Figs 10 and 11 report the dependence of loss modulus or static load at two different frequencies, 1 and 10 Hz. Here the dual behaviour of the natural ligaments and tendons are easily inferred by the crossing of the two curves: lower static stress

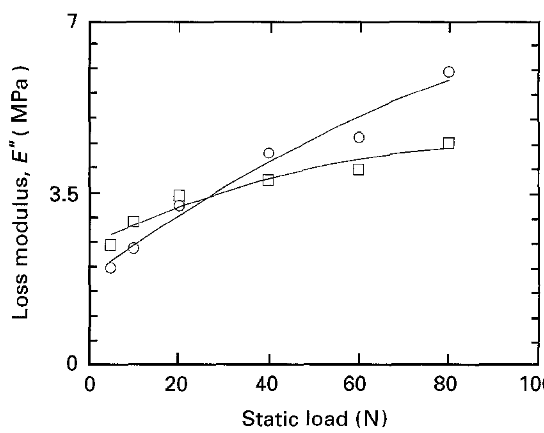

Figure 10 Loss modulus versus static load for ACL at dynamic load 5 N: —○— 1 Hz; —□— 10 Hz.

means rubber-like behaviour (increase of loss modulus with frequency), higher static load means glassy-like behaviour (decrease of loss modulus with frequency). In the case of tendons the crossing point occurs at a lower value of load (16 N) than for the ligament (28 N). This confirms the different tensile properties and structure of the two systems: the mechanical contribution of collagen fibre in tendon occurs earlier than in ligament. In fact, the collagen fibres in the tendon have a unidirectional waveform arrangement,

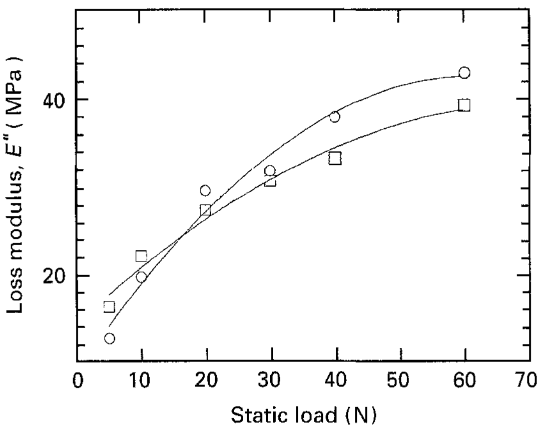

Figure 11 Loss modulus versus static load for Achilles' tendon at dynamic load 5 N: —○— 1 Hz; —□— 10 Hz.

while in the ligament collagen fibres are in waveform and oriented in different directions, and organized in two separated bundles.

5. Discussion

The observed behaviour can be explained in terms of the intrinsic morphology of the connective tissues, which can be represented as an interacing network of collagen fibres embedded in a proteoglycans matrix. The imposition of external load induces distortion of this microstructural disordered network, which responds by reorienting the fibrous phase along preferential directions to a greater or lesser degree. The mechanical behaviour depends strictly on the correlation between the configurational changes of the microstructure and the macroscopically observed deformation. The typical waveform arrangement of collagen bundles causes a progressive transition between states of stretching of the specimen with different numbers of effectively loaded bundles. At low load, only a few collagen bundles are effectively loaded and the mechanical response of the specimen can be mainly attributed to the properties of the proteglycans matrix. This is evident from the toe region of the static curve (low modulus region) and from the rubber-like behaviour highlighted by the dynamic-mechanical analyses. Conversely, at high strain most of the collagen bundles are almost completely extended and the mechanical response of the tissues is governed by the properties of the collagen fibres. This becomes evident from the "linear" region of the static curve (high modulus region) and from the glassy-like behaviour evidenced by the decrease of loss modulus with frequency (Figs 10, 11).

These properties of the tissue are very important in explaining their toughness and shock-absorbing features. It is worth noting that most common monolithic engineering materials exhibit a convex stress–strain curve and do not show any rubbery–glassy transition under static load. The uncommon shape of the stress–strain curve and viscoelastic properties shown by these natural structures are due to their composite structure. The presence of an initial low modulus region allows the tissue to deform with very low strain energy, thus explaining the greater toughness of the biological tissues when compared to engineering materials having fracture work of the same order of magnitude.

The transition from a rubber-like (increase of storage and loss modulus with frequency) to a glassy-like behaviour (increase of storage modulus and decrease of loss modulus) is typical of polymer-based composite structures.

6. Conclusions

The viscoelastic analysis performed in this study revealed the relationship between structure and mechanical properties of natural tendons and ligaments. The proteoglycans, elastin, water, and collagen fibres play different mechanical roles: the low modulus proteoglycans matrix is responsible for the low strain mechanical behaviour, whereas the high modulus collagen fibres govern the high strain behaviour.

The overall mechanical behaviour is characterized by a gradual transition from the low modulus region (low strain) to the linear region (high strain). The transition occurs through the sequential stretching of initially crimped fibre bundles. The static properties of the tissue mainly depend on the orientation degree of these bundles with respect to the loaded axis. The dynamic behaviour also depends on the kinematics of the collagen fibres orientation. As the deformation rate increases, the elastic modulus slightly increases due to a progressive reduction of the rate of collagen fibres rearrangement. The increasing difficulty of the collagen fibres to rearrange themselves in the gel matrix as the deformation rate increases, leads to a reduction of loss moduli of the tissue at higher static load. At low static load (low strain), higher deformation rate means higher inelastic work (increase of loss modulus), whereas at high static load (high strain) a higher deformation rate implies lower inelastic work (decrease of loss modulus).

The results obtained in this work provide useful information for the design of more appropriate prostheses, and indicate that composite structures are the best materials to emulate the mechanical behaviour of natural tendons and ligaments.

References

1. A. HILTNER, J. J. CASSIDY and E. BAER, *Ann. Rev. Mater. Sci.* **15** (1985) 455–482.
2. J. D. C. CRISP, Jr, in "Biomechanics, its foundations and objectives", edited by Y. C. Fung, N. Perrone and M. Anliker (Prentice-Hall, Englewood Cliffs, NJ, 1972) pp. 141–179.
3. F. G. GIRGIS, J. L. MARSHALL and A. R. S. AL MONAJEM, *Clin. Orthop. Rel. Res.* **106** (1975) 216–231.
4. A. A. AMIS and G. P. C. DAWKINS, *J. Bone Jt Surg. (Br)*, **73-B** (1991) 260–267.
5. D. AMIEL, C. FRANK, F. HARWOOD, J. FRONEK and W. AKESON, *J. Orthop. Res.* **1** (1984) 257–265.
6. S. P. ARNOCZKY, J. R. MATYAS, J. A. BUCKWALTER and D. AMIEL, in "The anterior cruciate ligament: current and future concepts", edited by D. W. Jackson, S. P. Arnoczky, C. B. Frank, S. L.-Y. Woo and T. M. Simon (associate editors) (Raven Press, New York, 1993) Ch. 1, pp. 5–22.

7. J. D. FERRY, "Viscoelastic properties of polymers" (Wiley, New York, 1980) Third Edition, Ch. 3, pp. 56–79.
8. R. M. CHRISTENSEN, "Theory of viscoelasticity: an introduction" (Academic Press, New York, 1971).
9. T. MURAYAMA, "Dynamic mechanical analysis of polymer materials" (Elsevier, New York, 1978).
10. W. C. HERRICK, H. B. KINGSBURRY and D. Y. S. LOU, *J. Biomed. Mater. Res.* **2** (1978) 877–894.
11. S. L.-Y. WOO, *Biorheology* **19** (1982) 385–396.
12. J. B. PARK, "Biomaterials: an introduction" (Plenum Press, New York, 1979) Ch. 7, pp. 97–130.
13. R. C. HAUT, in "The anterior cruciate ligament: current and future concepts", edited by D. W. Jackson, S. P. Arnoczky, C. B. Frank, S. L.-Y. Woo and T. M. Simon (associate editors) (Raven Press, New York, 1993) Ch. 5, pp. 63–73.
14. M. K. KWAN, T. H.-C. LIN and S. L.-Y. WOO, *J. Biomechanics* **26** (1993) 447–452.

Received 4 May
and accepted 5 May 1995