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The human anterior cruciate ligament: histological and ultrastructural observations

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ABSTRACT

In transverse and longitudinal paraffin-embedded sections, the human anterior cruciate ligament (ACL) is made up of wavy bundles of collagen fibres arrayed in various directions, the majority around the axis of the ligament with a few running parallel to it. The fascicles making up the larger bundles are also characterised by this undulating appearance. In thin sections 2 types of collagen fibrils are observed: small (with a single diameter peak at 45 nm) and large (3 peaks at 35, 50 and 75 nm respectively), organised into distinct areas made up of either large or small bundles of fibrils. The numerous fibroblasts that are present appear elongated in the direction of the bundles with branches and short cytoplasmic processes. The elastic system is made up of both elastic and oxytalan fibres. The varied orientation of the bundles in the ACL, the complex ultrastructural organisation and the abundant elastic system make it very different from other ligaments and tendons, providing a structure able to withstand the multiaxial stresses and varying tensile strains imposed upon it.

INTRODUCTION

Although widely investigated, the anterior cruciate ligament (ACL) is still a highly pertinent area for study. It is often injured during strenuous exercise and heals with difficulty, frequently impairing athletic performance. Secondly, despite the numerous exhaustive biomechanical studies on the ACL (Kennedy et al. 1974; Butler et al. 1985; Dahhan, 1985; Ellison & Berg, 1985; McLeod, 1985; Odensten & Gillquist, 1985; Gollehon et al. 1987), the morphological and structural descriptions of the literature are concerned only with its macroscopic features and the appearances on light microscopy. Some authors (Kennedy et al. 1974; Arnoczky, 1983; Butler et al. 1985) have described the ACL as a multifascicular structure whose individual bundles, each ensheathed in loose connective tissue, either spiral around or lie along the axis of the ligament. Odensten and Gillquist (1985) described the ACL as a homogeneous structure, while Danylchuk et al. (1978), using scanning electron microscopy, reported a more complex structure where straight collagen bundles are formed by a 'complex network of interlacing fibrils'.

The biomechanical data are also discordant. Although there is general agreement on the ability of the ACL to maintain homogeneous tension during flexion–extension and knee rotation (Noyes & Grood, 1976; Butler et al. 1985; Dahhan, 1985), Sapega et al. (1990) pointed out that these isometric bundles comprise only a small area of the whole ligament.

In the light of the somewhat discordant and incomplete morphological data in the literature, this study investigated the ultrastructural aspects of the human ACL, with special attention to the size, arrangement and distribution of the collagen fibrils and of the elastic system in an attempt to provide new insight into the biomechanics of this ligament.

MATERIALS AND METHODS

The ACL from 15 patients (age range 45–87 y) was obtained during leg amputation because of traumatic injury (8 cases), ilial chondrosarcoma (3 cases),

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fibrosarcoma (2 cases) and osteosarcoma (2 cases). In all the cases, the ACLs showed no signs of involvement. Specimens were processed for light and transmission electron microscopy.

Samples for light microscopy were clamped at both ends to avoid shrinkage and then fixed in 10% formalin, dehydrated and embedded in paraffin. Serial sections (5 μm) were stained with haematoxylin-eosin, Mallory's azan and Picro-Sirius Red. Elective Weigert's resorcin-fuchsin and resorcin-fuchsin staining of previously oxone oxidised material (Fullmer & Lillie, 1958) was employed to distinguish elastin and elaunin fibres from oxytalan fibres. The samples for electron microscopy were fixed in 2.5% glutaraldehyde and 4% formaldehyde with 0.1% tannic acid in 0.1 M cacodylate buffer, pH 7.4. The blocks were postfixed in 1% osmium tetroxide diluted in the same buffer, dehydrated in alcohol and embedded in Araldite. The thin sections were contrasted with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 1A electron microscope.

Transversely sectioned areas of collagen fibrils were examined in thin sections with a Leitz ASM image analysis computer system, randomly selected from different portions of the ligament, after checking magnification using a grating replica with 2160 lines/nm. For each sample, measurements were taken for at least 5 photomicrographs. Collagen fibril diameters were obtained from the selected areas by the relation $D = \sqrt{\frac{4}{\pi}}$; volumetric density was expressed as total area of the fibrils divided by the sample area. In addition, the areas occupied by collagen fibrils, cells and the elastic components were assessed.

RESULTS

Longitudinal paraffin sections show that the ACL is composed of thick wavy bundles of collagen fibres ensheathed in loose connective tissue. Thin connective tissue processes course from the sheath into the bundles, subdividing these into a series of smaller fascicles. The latter appear to have an undulating course, arrayed in various directions (Fig. 1). Serial transverse sections stained with Picro-Sirius Red or Mallory's azan show an alternating fascicular array, at times transverse to the long axis of the ligament, then oblique and once again transverse, hence appearing as a multiaxial organisation. Thin transverse sections show the existence of 2 different types of collagen fibrils (Fig. 2). The first, which accounts for 50.3% of the whole ACL, shows a nonuniform diameter ranging from 25 to 85 nm and an irregular outline (Fig. 3). Their diameter distribution curve is characterised by 3 peaks; these are at 35, 50 and 75 nm (Fig. 4*a*). The fibrils of the second type, representing 43.7% of the whole ACL, have a uniform diameter and smooth margins (Fig. 3). Their diameter frequency is a tight gaussian curve, peaking at 45 nm (Fig. 4*b*).

Distributed between the collagen fascicles are many fibroblasts whose cell bodies appear elongated. Sections stained with Weigert's resorcin-fuchsin reveal

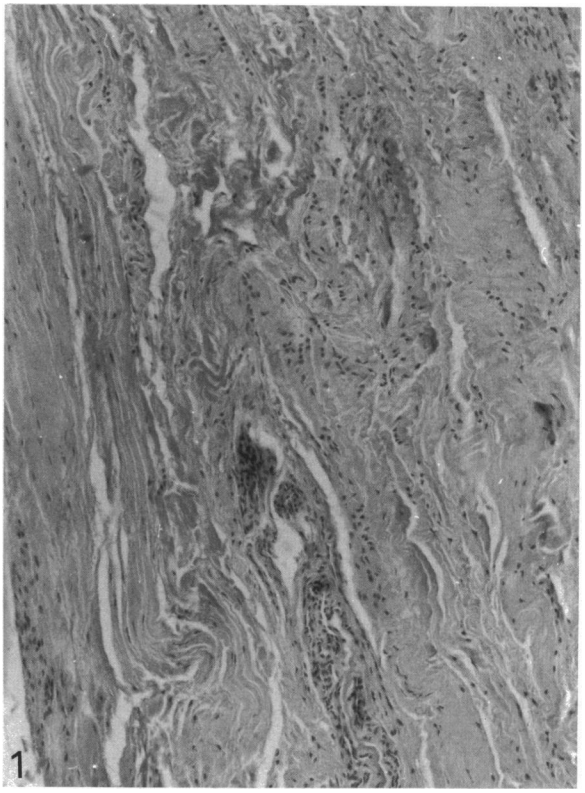

Fig. 1. Longitudinal paraffin section of a human ACL fibre bundle which appears to be subdivided into small undulating fascicles. Haematoxylin-eosin, × 116.

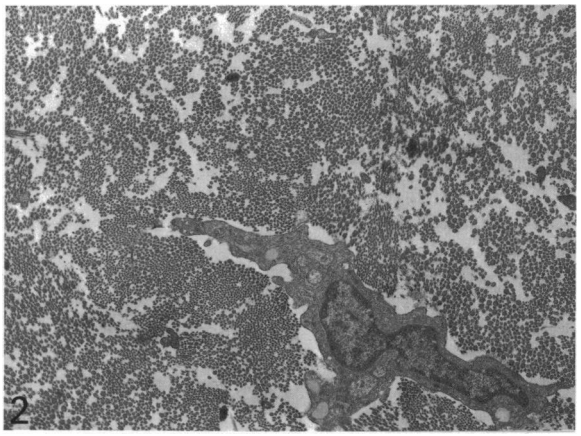

Fig. 2. Thin transverse section of ACL fascicles composed of an inhomogeneous population of collagen fibrils. An elongated fibroblast is observed between the collagen fascicles. × 7000.

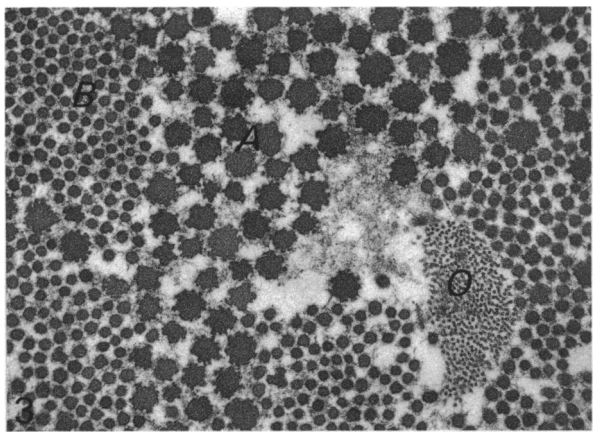

Fig. 3. Cross-sectioned collagen fibrils with a nonuniform diameter and irregular outline (*A*) and a uniform diameter and regular outline (*B*). *O*, bundle of oxytalan microfibrils. ×41000.

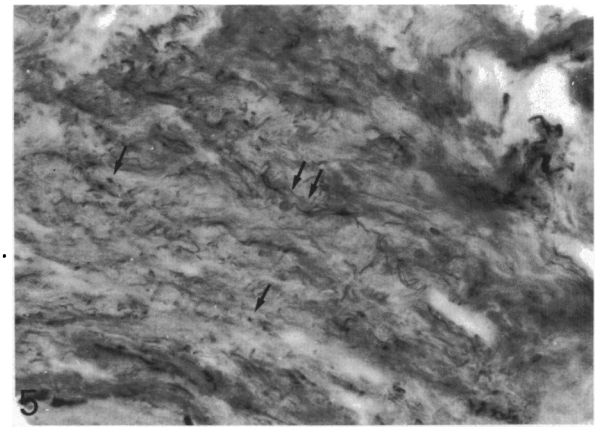

Fig. 5. Weigert's resorcin-fuchsin stained paraffin section which shows abundant elastic fibres both in transverse (single arrows) and longitudinal (double arrows) sections. ×600.

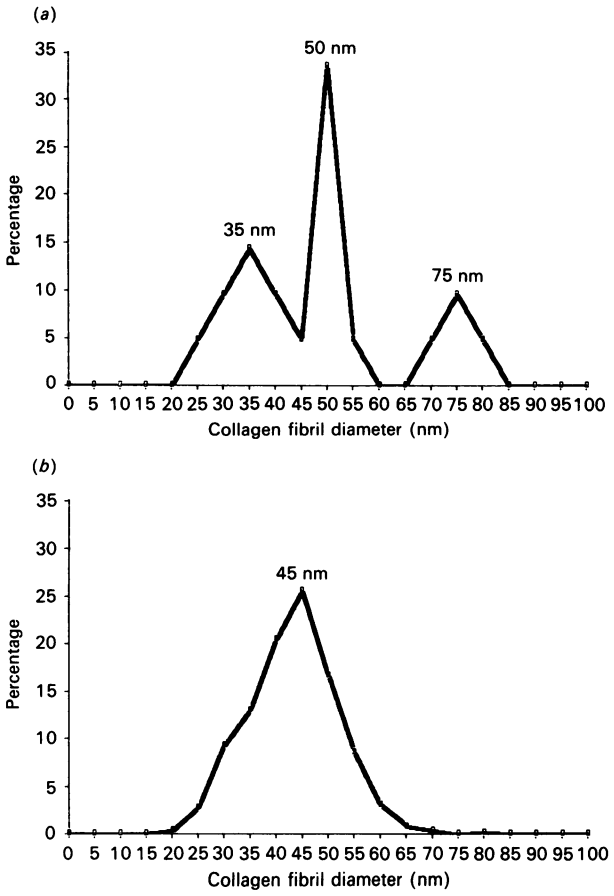

Fig. 4. Diameter distribution curves for (*a*) collagen fibrils with a nonuniform diameter (the curve is characterised by 3 peaks at 35, 50 and 75 nm, respectively) and (*b*) collagen fibrils with a uniform diameter (the curve is characterised by a single peak at 45 nm).

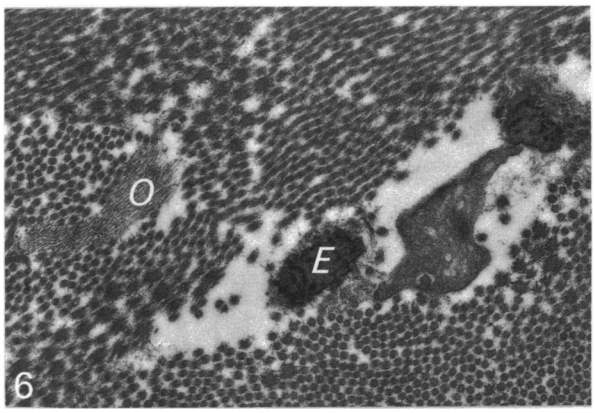

Fig. 6. Thin section showing elastic fibres (*E*) situated between 2 fascicles of collagen fibrils. An oxytalan fibre (*O*) is also present. ×20000.

the presence of abundant elastic fibres. Oxidation followed by resorcin-fuchsin staining (Fullmer et al. 1974) demonstrates numerous oxytalan fibres in areas where elastic fibres are absent. Both elastic and oxytalan fibres appear to be distributed along the individual bundles (Fig. 5). At the ultrastructural level elastic fibres present a central amorphous region surrounded by many 10–12 nm microfibrils (Fig. 6). Bundles of microfibrils which in longitudinal sections have a beaded appearance were interpreted as oxytalan fibres. In transverse sections the diameter of these microfibrils averages 10–12 nm and their central core appears modestly electron dense (Fig. 3). Cells and elastic components account for 6% of all ACL tissues.

DISCUSSION

The histological and ultrastructural appearances of the ACLs examined differ from those of other ligaments and tendons, opening up discussion as to the mechanical interpretation to be given to these structures. The collagen fibre bundles lie in many directions and the ultrastructural organisation is varied and complex. Abundant elastic tissue is present. The serial sections show that the majority of fascicles are oriented at various angles to the long axis of the

ligament, while only a few fascicles run parallel to this axis. All these fascicles were in the resting position. Moreover ultrastructural examination shows 2 types of fibrils, the first having a variable diameter and irregular outline, the second a uniform diameter and smooth profile.

Fibril diameter is known to indicate specific fibril function (Flint et al. 1984). For example, collagen fibril diameter is known to vary with age in certain tissues, suggesting a correlation with functional status. The progressive increase in average collagen fibril diameter during development entails an increase in the number of intermolecular cross-links, which in turn enhances the tensile strength of the tissue in keeping with the greater functional demands made upon it (Nimni, 1983). Furthermore, tight bundles of collagen fibrils of markedly differing diameters are known to be a prominent feature of tendons and reticular dermis, i.e. tissues whose main function is to resist stretching. Conversely, smaller collagen fibrils of uniform diameter are present in tissues subjected to multidirectional stresses (Raspanti et al. 1990), such as walls of vessels and hollow viscera, fibrous sheaths and parenchyma.

It is therefore reasonable to assume that the different collagen fibril groups observed have differing functions, the larger inhomogeneous fibrils being specialised for resisting high tensile stresses, with the small homogeneous fibrils maintaining the 3-dimensional organisation of the ligament. It would follow that the structure of the ACL is very different from other ligaments and tendons, as indeed previously proposed by some authors (Danylchuk et al. 1978; Arnoczky, 1983). It should be noted, however, that the collagen fibril diameters reported by these authors differ markedly from those obtained in the present study (150–250 nm against a range of 25–85 nm). The numerous oxytalan and elastic fibres interspersed among the different collagen fibril areas may be presumed to mediate between the different functional roles of the collagen fibrils. Oxytalan and elastic fibres have different functional implications. Oxytalan fibres, present in soft tissues such as tendon sheaths (Caldini et al. 1990), periodontum (Fullmer & Lillie, 1958) and the dermoepidermal junction (Cotta-Pereira et al. 1976), are particularly suited to withstand modest multidirectional stresses, while elastic fibres which occur in more rigid tissues such as tendons and ligaments (Cooper & Misol, 1970; Beckman & Greenlee, 1975; Oakes & Bialkower, 1977) and the human vocal cord (Hirano, 1974; R. Strocchi, unpublished data), absorb recurrent maximal stress. From the mechanical standpoint this system of fibril bundles, which is presumably arrayed to offset multidirectional tensile stresses, appears to form an isotropic structure, specifically designed to allow homogeneous distribution of multiaxial stresses. In fact, the various combinations of mechanical stresses to which the ACL is subjected, could hardly be supported by a simple series of isometric linear bundles.

In conclusion, the ACL has been observed to be a complex anatomical structure, indeed an isotropic system capable of providing varying tensile resistances to the multiaxial stresses to which it is subjected.

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