

Collagen Fibril Orientations in Tissues and their Relationship to Mechanical Properties

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Introduction

Collagen fibrils provide tensile reinforcement to the extracellular matrix (ECM) of tissues. The mechanical properties of connective tissues, such as tendon, cartilage etc., depend on their ECM being able to withstand appreciable applied forces. These forces may be generated by the musculature or be externally applied. Collagen molecules are rod-shaped and packed together to form fibrils which are stabilised by intermolecular covalent cross-links. Thus the fibrils are stiff and strong when they are subjected to axial tension but have little flexural, torsional or compressive stiffness, except in hard tissues like bone where the mineral phase modifies the properties of collagen.

Therefore, collagen fibrils can reinforce soft tissues provided that they are oriented in the directions in which tensile stress is generated. Thus the ECM is a biological example of a fibre-reinforced composite material. However, many tissues have to withstand compression, torsion and flexion as well as tension. Collagen fibrils can still provide reinforcement provided they are oriented so that they are placed in tension when the tissue is loaded. Provided the directions in which the fibrils are oriented can be measured experimentally, the results can be identified with the directions in which the tissue can withstand tensile stress and used to formulate models for the mechanical stability of tissues. This approach has been applied to understanding the mechanical stability of articular cartilage [1], the meniscus of the knee joint [2], the urethra [3], the intervertebral disc [4], spinal ligaments [5] and the uterine cervix [6]. More recently, it is being used to investigate the structural basis of creep and stress relaxation in ECM [7].

Determination of fibril orientation

The most convenient method for determining the orientation of collagen fibrils in a tissue site is from a single high-angle X-ray diffraction pattern. The method depends on the ability to obtain a tissue sample, which may be a section cut for the purpose, in which the preferred orientation of the fibrils is in a plane perpendicular to the X-ray beam. The equator of the diffraction pattern then defines the perpendicular to the preferred orientation and the angular spread of the equatorial intensity maxima depends on the orientation distribution function, $g(\phi)$, defined to be the probability of finding a fibril oriented between ϕ and $\phi + d\phi$ with respect to the preferred orientation [8].

If $I(\phi)$ is the angular distribution of equatorial intensity from a perfectly oriented sample (a dogfish fin-ray), then the corresponding intensity distribution from an imperfectly oriented assembly of fibrils is given by

$$I_s(\phi) = g(\phi) * I(\phi)$$

where $*$ represents convolution. The most convenient means of recovering $g(\phi)$ from experimental measurements of $I(\phi)$ and $I_s(\phi)$ is to express them as sums of even-ordered Legendre polynomials [8] of the form

$$I(\phi) = \sum_{n=0}^{\infty} (n + 1/2) \langle P_n \rangle P_n(\cos \phi)$$

where the summation is over sufficient even orders of n to reproduce the experimentally determined form of $I(\phi)$ and $\langle P_n \rangle$ are empirical coefficients required to obtain a satisfactory fit. The Legendre polynomial $P_n(\cos \phi)$ is completely defined in the range $0 \leq \phi \leq 2\pi$ and can be computed using the Rodrigues' formula

$$P_n(x) = \sum_m^M (-1)^m (2n - 2m)! x^{n-2m} / \{2^n m!(n-m)!(n-2m)!\}$$

formula

where $M = n/2$ for n even. Values of $\langle P_n \rangle$ can be computed from the experimentally determined $I(\phi)$

$$\langle P_n \rangle = \frac{\int_0^\pi I(\phi) P_n(\cos \phi) \sin \phi d\phi}{\int_0^\pi I(\phi) \sin \phi d\phi}$$

by

The orientation distribution function, $g(\phi)$, may then

$$g(\phi) = \sum_{n=0}^{\infty} (n+1/2) \langle P_n \rangle_g P_n(\cos\phi)$$

be computed from

$$\langle P_n \rangle_g = \langle P_n \rangle_s / \langle P_n \rangle$$

where

Here $\langle P_n \rangle_s$ are the empirical coefficients determined from $I_s(\phi)$, the angular intensity distribution from the tissue site, and $\langle P_n \rangle$ are determined from $I(\phi)$, the intensity distribution from the fin-ray.

Compression of the intervertebral disc

The response of the intervertebral disc to compression will be used to illustrate how collagen fibril orientations can be used to formulate models for tissue stability and how such models may be tested experimentally. The intervertebral disc forms a flexible coupling between the vertebrae of the spine [4]. It is roughly cylindrical and has a soft centre, the nucleus pulposus, in which the collagen fibrils are apparently randomly oriented. The nucleus pulposus is surrounded by the annulus fibrosus which consists of a series of coaxial lamellae. In a single lamella the collagen fibrils are highly oriented at an angle of about 65° with respect to the axis of the spine; the direction of tilt alternates in successive lamellae. Figure 1 shows a schematic diagram of a single collagen fibril in a single lamella. The fibril has length, L , tilt, θ , and azimuthal span, ω radians. The lamella has height, h , and radius, r , and encloses a

$$V = \pi r^2 h = \pi [(L/\omega) \sin\theta]^2 L \cos\theta = (\pi/\omega^2) L^3 \sin^2\theta \cos\theta$$

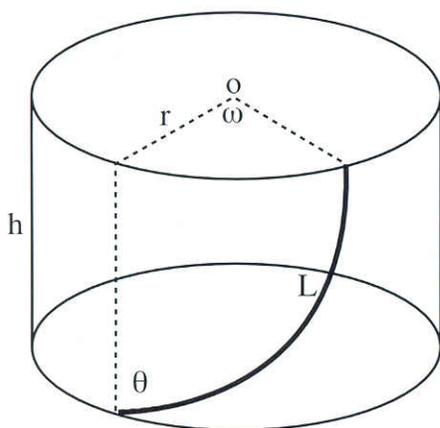


Figure 1. Schematic diagram of the path of a single collagen fibril (bold) in a lamella of the annulus fibrosus of the intervertebral disc. The lamella is modelled as a cylinder of radius, r , and height, h , whose upper face is centred at O . Then ω is the azimuthal span of the fibre which is also characterised by a tilt, θ , and length, L .

volume

$$\delta V = (\partial V / \partial L) \delta L + (\partial V / \partial \theta) \delta \theta$$

$$= 3(\pi/\omega^2)(L^2 \sin^2\theta \cos\theta) \delta L + (\pi/\omega^2)L^3(2 \sin\theta \cos^2\theta - \sin^3\theta) \delta \theta$$

$$\Rightarrow \delta V / V = (3/L) \delta L + (2 \cos^2\theta - \sin^2\theta) \delta \theta / (\sin\theta \cos\theta)$$

For pure compression, ω remains constant, and for compression at constant volume, $\delta V / V = 0$. If the collagen fibrils are subjected to a tensile stress, they will acquire a tensile strain so that $\delta L / L > 0$. This condition will be satisfied if $\sin^2\theta > 2 \cos^2\theta$, *i.e.* if $\theta > 54.7^\circ$.

Since experiment shows that $\theta \approx 65^\circ$, the collagen fibrils are strained when the disc is compressed, *i.e.* the fibrils provide effective tensile reinforcement. If radius is constant, simple geometry also shows that when the disc is compressed and the fibrils are strained, the value of θ increases from its initial

$$\theta = \tan^{-1}[(h_0/h) \tan\theta_0]$$

value, θ_0 , to

where h_0 is the initial value of the disc height, h . This relationship has been used to test the theory of the response of the collagen fibril network of the annulus fibrosus to compression of the intervertebral disc; a fully hydrated intact rabbit disc was compressed in stages and X-ray diffraction patterns were recorded at each stage to show that the fibre tilt increased as predicted [9]. More complicated finite element models show how the annulus fibrosus bulges as the disc is compressed [10]. In the living spine, the disc is often subjected to offset compression which leads to bending. Magnetic Resonance (MR) images of human volunteers then shows that the nucleus pulposus shifts within the disc [11].

Viscoelasticity

High-angle diffraction patterns from connective tissues have usually been obtained using conventional X-ray sources. In some experiments the tissue has been subjected to a series of fixed strains. A diffraction pattern has then been obtained from each strained state with a typical exposure time of about 30min. However, most tissues are viscoelastic. For example, the viscoelastic properties of both the intact intervertebral disc [12] and the nucleus pulposus [13] have been established. A viscoelastic material dissipates some of its strain energy. Since any dissipation process has a finite relaxation time, the stiffness of a viscoelastic material decreases with decreased loading rate. Thus, when a tissue is

subjected to a fixed strain, the stress within the tissue decreases, *i.e.* it experiences stress relaxation. Similarly, when a tissue is subjected to a fixed stress its strain will continue to increase, *i.e.* it exhibits creep.

Recent experiments have used synchrotron radiation to investigate the orientation of collagen fibrils in skin and perimysium (muscle connective tissue) [7]. In both tissues, the initial application of load leads to fibrils tending to reorient in the direction of applied load. However, there was no detectable change in fibril orientation during either creep or stress relaxation. These results indicate that simple geometric models are inadequate to explain the viscoelastic properties of ECM. This result is expected for stress relaxation, where the overall dimensions of the tissue do not change, and is consistent with X-ray diffraction patterns of intervertebral disc [4] and ligaments [5] showing fixed fibril orientations over a period of about 30 min at fixed strains. However, the results of creep experiments are more surprising and merit further investigation.

Preliminary experiments are also being performed on the response of the collagen fibril network of uterine cervix to creep (in collaboration with Mr S.J. Wilkinson). These experiments are being performed on rat tissue which provides an established model for the changes which occur in the human cervix during labour [6]. The creep rate of the cervix increases at term, allowing it to dilate so that the neonate can pass through. Furthermore, this change in mechanical properties is accompanied by a change in NMR relaxation times [14] which may explain the changes apparent in MR images of human patients. Understanding the relationship between structure and mechanical properties of the the rat cervix may then be a step in relating the appearance of the human cervix in MR images to its properties and, hence, to the diagnosis of a cervix which changes its mechanical properties too early in pregnancy or not at all.

References

[1] D.W.L. Hukins, R.M. Aspden. In *Material Properties and Stress Analysis in Biomechanics*, A.L. Yettram (ed.), pp. 44-59, Manchester University Press, Manchester, 1989.

- [2] R.M. Aspden, D.W.L. Hukins. In *Material Properties and Stress Analysis in Biomechanics*, A.L. Yettram (ed.), pp. 109-122, Manchester University Press, Manchester, 1989.
- [3] D.S. Hickey, J.I. Phillips, D.W.L. Hukins. *British Journal of Urology* **54**, 556-561 (1982).
- [4] D.W.L. Hukins. In *Biology of the Intervertebral Disc*, vol. 1, P. Ghosh (ed.), pp. 1-37, CRC Press, Boca Raton, 1988.
- [5] D.W.L. Hukins, M.C. Kirby, T.A. Sikoryn, R.M. Aspden, A.J. Cox. *Spine* **15**, 787-795 (1990).
- [6] R.M. Aspden. In *Connective Tissue Matrix*, part 2, D.W.L. Hukins (ed.), pp. 199-228, Macmillan, London, 1990.
- [7] P.P. Purslow, D.W.L. Hukins. International Biomechanics Conference, Amsterdam, 1994.
- [8] M.C. Kirby, R.M. Aspden, D.W.L. Hukins. *Journal of Applied Crystallography* **21**, 929-934 (1988).
- [9] J.A. Klein, D.W.L. Hukins, *Biochimica et Biophysica Acta* **717**, 61-64 (1982).
- [10] K.J. Mathias, J.R. Meakin, A. Heaton, M.W. Brian, S. Mierendorff, R.M. Aspden, J.C. Leahy, D.W.L. Hukins. In *NAFEMS World Congress*, in press.
- [11] A.J. Fennell, A.P. Jones, D.W.L. Hukins. *Spine*, in press.
- [12] A.D. Holmes, D.W.L. Hukins. *Medical Engineering and Physics* **18**, 99-104 (1996).
- [13] J.C. Leahy, D.W.L. Hukins. *Journal of Back and Musculoskeletal Rehabilitation*, in press.
- [14] J. Blacker, M.A. Foster, R.M. Aspden. British Institute of Radiology Meeting, Birmingham,

Diffraction by Disordered Fibres

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Introduction

Diffraction patterns from some polycrystalline fibres contain sharp Bragg reflections at low resolution, giving way to continuous layer line intensities at high resolution [1,2]. Such specimens are essentially polycrystalline, but the packing of the molecules in the crystallites is disordered. Accurate structure