NON-DESTRUCTIVE OPTICAL MONITORING OF EXTRACELLULAR MATRIX ORGANISATION IN TENDON

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INTRODUCTION: Tendons are highly orientated (anisotropic) fibrous structures adapted for a high load bearing mechanical function.[2] As a tissue engineered tendon construct matures *in vitro*, it is vital to be able to non-invasively monitor its structural development. Elastic Scattering Spectroscopy (ESS) is a novel, low cost, non-invasive technique which delivers white light through an optical fibre to the surface of a material.[1] The pattern of absorption and back scattering of light detected gives real time, quantitative information of structure allowing *in situ* monitoring. However, interpretation depends on understanding responses in normal tendon.

METHODS: Hind paw flexor tendons from New Zealand white rabbits were examined *in situ* using ESS (with a source/detector separation of 300μm). Spectra were obtained in the 350 – 750nm range with tendons in resting position and during passive stretch, i.e. full range of limb motion. Probe was orientated 1) longitudinally (in line with tendon fibre orientation) and 2) perpendicular to the tendon long axis. 12 tendons from 3 animals were tested to identify inter- and intra-tendon differences.

RESULTS: All the spectra obtained had a similar and characteristic appearance with minimal variability between tendons (Fig 1).

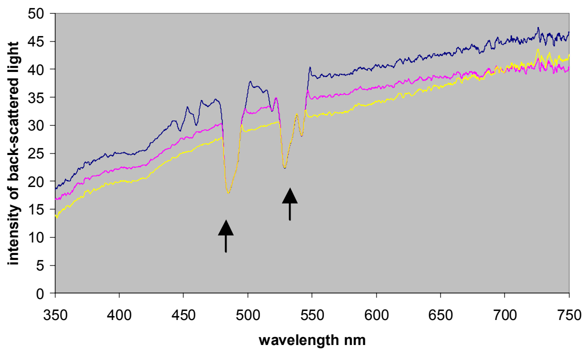

Fig.1: Characteristic spectra from different tendons in relaxed state (arrows show light absorption by haemoglobin)

Spectral signatures at 600nm were analysed with the probe positioned longitudinal & perpendicular to the tendon long axis, hence collagen fibre alignment (Fig. 2). In all specimens there was an alignment of collagen fibrils under load, which could be detected by an increase in optical anisotropy. This anisotropic effect became enhanced with passive stretch of the tendons as the limb position was moved.

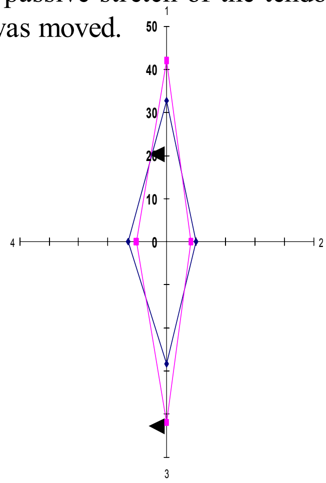

Fig. 2: (— resting position — passive stretch). Axes show arbitrary scale for intensity of back scattered light detected. Probe angle from 0°-90-180-270° (points 1-4) along tendon long axis. As tendon is stretched and collagen fibres align, increased back-scattered light is detected in the longitudinal direction than the transverse. Note: Significant change in shape of graph (arrows).

DISCUSSION & CONCLUSIONS: During mechanical loading, tendon matrix undergoes structural change, with loss of crimp and compaction of collagen fibres.[3] We have analysed and characterized these changes in situ using ESS to identify increased anisotropy (Fig 2). We conclude that ESS is a useful method for monitoring changes in collagen orientation during loading. As such, this technique has major potential for non-invasive monitoring (real time) of structural changes in tissue-engineered constructs.

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