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RHEOLOGICAL PROPERTIES OF ANISOTROPIC TISSUES AT LARGE AMPLITUDE OSCILLATORY SHEAR

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INTRODUCTION

The mechanical properties of soft biological tissues have been widely investigated over the past five decades [1-5]. Reported measurements of soft biological tissues such as the brain, spinal cord, liver and muscle vary by orders of magnitude, depending on the sample preparation, anisotropy and loading regime. Knowing the accurate mechanical properties of biological tissues is important for many applications, for example car crash testing and simulations require accurate information on how different parts of the body deform due to a combination of loads. Deformation of tissues around prosthetics and artificial limbs are critical in understanding load transfer at interfaces with the body. The recent use of Magnetic Resonance Elastography (MRE) in diagnostic imaging has resulted in a surge of interest in accurate measurements of mechanical properties of tissues [6].

A transition is observed in the stress response of material when the applied amplitude of an oscillatory shear strain is increased beyond the linear viscoelastic regime. We refer to this as Large Amplitude Oscillatory Strain (LAOS). Beyond the linear viscoelastic limit (LVL), the shear storage and loss moduli (G' and G" respectively, calculated by assuming that the stress response is sinusoidal to a sinusoidal input strain) are difficult to interpret, and should not be used to describe the material's viscoelasticity [4]. Therefore, investigating tissue viscoelasticity using oscillatory methods has been largely limited to the linear viscoelastic regime or wrongly characterised using linear methods even though the material was being loaded beyond the LVL.

Fourier Transformation rheology (FT-rheology) is a new method of characterising LAOS data by decomposing the stress output waveforms into harmonic components using Fourier analysis [7]. This decomposition can then be used to estimate the harmonic components of both the storage and loss moduli.

METHODS

A rotational rheometer (Kinexus Pro KNX 2100, Malvern, United Kingdom) was used to measure the properties of bovine skeletal muscle. A pair of 40 mm diameter serrated plates were used and the samples were immersed in 0.9% sodium chloride solution prior to testing [8]. Samples were 10 mm in diameter and 3 mm thickness. 8 pre-conditioning cycles was applied prior to each test [9]. The temperature was controlled to 25°C and strain sweep tests were conducted at a frequency of 1 Hz and pre-compressive strain of 10%.

The samples were placed eccentrically at the edge of the plate to increase the measured torque and to obtain an approximately homogenous deformation [10-11]. The eccentric configuration allowed three fiber direction alignments to be investigated, denoted as Perp, Para1 and Para2 as shown in Figure 1. FT-rheology was used to investigate the deformation of tissue at LAOS by obtaining the decomposed harmonic components of G' and G" [7, 12-13].

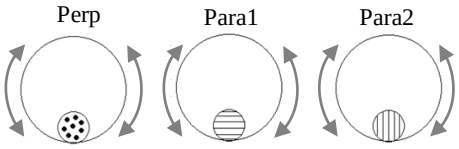

Figure 1: Schematic of fiber alignments for eccentric setup, arrows denote direction of shear.

RESULTS

The mean G' and G" as well as the shear linear viscoelastic limit for the three fiber alignments are itemized in Table 1. A statistical significant difference ($p<0.05$) of G' using ANOVA (N=6 for each fiber alignment) was found between the three fiber alignment groups.

Table 1: Summary of results within the linear viscoelastic regime.

Fiber Alignment	G' (Pa)	G'' (Pa)	LVL (%)
Perp	4,290	1,015	0.039
Para1	3,663	822	0.077
Para2	1,890	460	0.049

The first and third harmonic components of the shear storage modulus, G'_1 and G'_3 respectively, were decomposed using FT-rheology and are shown in Figure 2 and Figure 3. The first and third harmonic components were analysed because even harmonic components have been found to be related to transient responses, secondary flows [14] or dynamic wall slip [15] and higher harmonic components (fifth and above) were negligible.

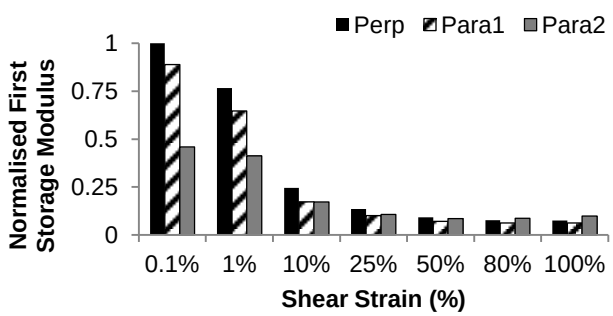

Figure 2: Normalised First Storage Modulus for shear strain spectrum.

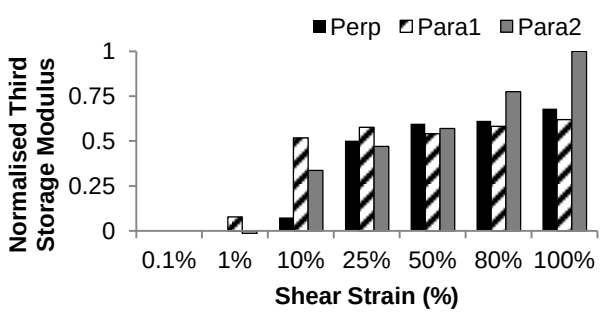

Figure 3:Normalised Third Storage Modulus for shear strain spectrum.

DISCUSSION

The viscoelastic properties of skeletal muscle are dependent on the shear loading direction to the muscle fibers and the underlying extracellular matrix. The varying non-linear response of the material properties is likely caused by the complex changes in the underlying microstructure and this is demonstrated by the appearance of higher harmonic components.

In the linear regime, it was found that G'_1 of the tissue was highest when shear strain was applied to the samples in the Perp direction. This is expected as the fibers were aligned to resist the shear applied to the sample thus resulting in a higher stiffness compared to samples placed in the parallel direction.

In the linear viscoelastic regime, the differences in G'_1 is likely due to tissue anisotropy and this decreases with increasing strain and plateaus from shear strains of 10%. In addition, Figure 3 shows the appearance of higher harmonic components from shear strains of 10%. Further investigation into the nonlinear response of G'_3 shows that this parameter is significantly different for Para2 samples at shear strains of 10%, 25%, 50%, 80% and 100%. In addition, G'_3 increases significantly with increasing strain for Perp and Para2 samples and this suggests a unique alteration in the underlying microstructure arrangements when mechanical loading is applied in these directions.

Further studies will be conducted to match microstructural changes in the tissue samples to noticeable deformation in Lissajous-Bowditch curves and harmonic component responses thus giving physical meaning to the characteristics of the unique mechanical response of skeletal muscles.

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