Correlative nano- to whole-joint-scale strain measurements in the intervertebral disc using TomoSAXS

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Possible Sessions

4. Biomaterials & Biomechanics, 16. Novel experimental techniques, 27. Tomography & Radiography

Introduction

Fibrous musculoskeletal tissues play a vital biomechanical role across biological systems. The function of these tissues depends on structural features from the nano- to whole-organ-scales. In the intervertebral disc (IVD), collagen fibres in the annulus fibrosus (AF) form a complex lamellar structure which enables load transfer and flexibility throughout the spine. Lower back pain is the leading cause of years lived with disability globally [1], with the IVDs playing a key role in spinal health. The multiscale biomechanical function of the IVD is therefore of great clinical importance; however, techniques enabling the correlative measurement of strain fields in fibrous tissues at the nano-, micro-, and whole-joint-scales have not previously been achieved. *In situ* synchrotron computed tomography (sCT) combined with digital volume correlation (DVC) has previously been used to measure microscale strain patterns in three-dimensions (3D) across the IVD [2,3]. At the nanoscale, small angle X-ray scattering (SAXS) has been used to study the nanoscale mechanics of collagenous tissues, traditionally in 2D [4]. Here, we present ***TomoSAXS,*** a multimodal imaging method that combines these methods to reconstruct 3D structure and mechanics of fibrous tissues across the nano-to-micro scales. Using sCT data to inform SAXS analysis permits spatial deconvolution of SAXS scattering for individual collagen fibres, enabling the measurement of nanoscale properties on a per-fibre basis. Combining TomoSAXS with *in situ* mechanical testing of whole IVD and DVC enables correlative strain measurements at the whole joint, tissue, microscale fibre, and nanoscale fibrillar levels.

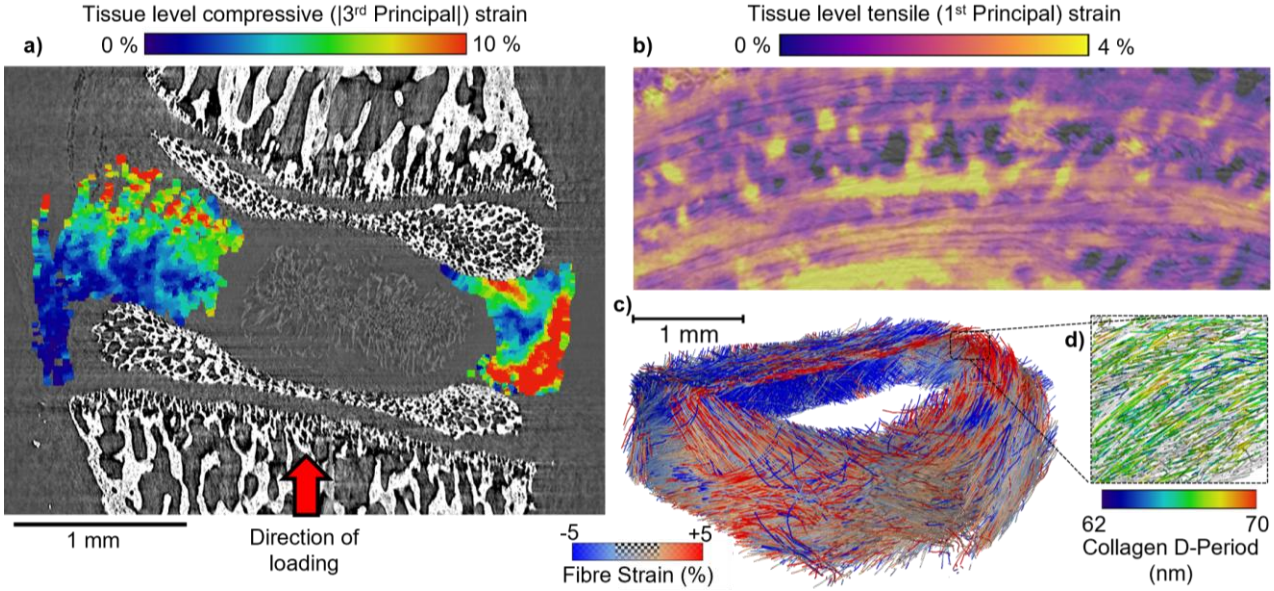

Figure 1 – a) Tissue level compressive strain in the AF mapped onto a greyscale sCT image of a rat IVD; b) tissue level tensile (1st principal) strain in the anterior AF; c) 3D rendering of fibre strain in the IVD; d) collagen D-period of fibres in the IVD.

Methods

In situ TomoSAXS (correlative sCT and SAXS) of fresh-frozen rat IVD (n=3) was performed at Diamond Light Source (DLS). SAXS scans (2D maps at 9 angles around one rotation axis) were performed at beamline I22 (voxel size 20 µm), sCT scans (voxel size 1.6 µm) were performed at I13-2. Two sets of scans were performed, the first with the sample held under a 1 N compressive preload, and the second after application of 50 µm compressive displacement. SAXS and sCT datasets were spatially aligned through co-registration of sample specific landmarks. Fibre tracing analysis of sCT data (Avizo XFiber 2023.2) was performed to measure collagen fibre bundle orientation in the AF, providing estimates of the orientation of fibres in each SAXS beampath. This information was fed into a simulation of the full SAXS tomography for

each sample, which was used to estimate angular (χ) regions of independent scattering of fibres in each beampath using 3D diffraction modelling [5]. These regions were sampled in three sequential angular sectors along the azimuthal (q) axis of SAXS frames to estimate the respective fibres nanoscale properties. Nanoscale properties, including collagen D-period (nm) (an indication of the level of strain in a collagen fibre) and fibril diameter (nm) were estimated by optimizing fits between diffraction models and sampled data. Deformation of the traced fibres was measured using DVC [2,3], and this data was used to calculate fibre orientations in the loaded scans for TomoSAXS reconstruction. Tissue level strain (Fig. 1a, b) was measured through polynomial fitting of the overall displacement field. Fibre level strain (Fig. 1c) was measured through weighted polynomial fitting to the displacement field along each fibre. Bulk IVD strain was calculated from the mean change in distance between the vertebral endplates, measured using DVC [2]. Tracking of fibres using DVC enabled change in nanoscale properties to be measured for each fibre (Fig. 2).

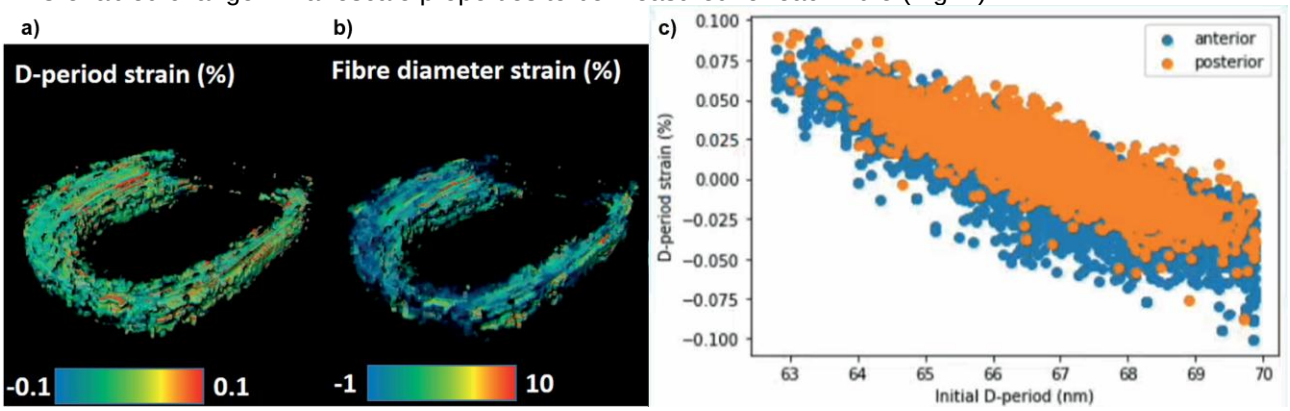

Figure 2 – 3D renderings of a) D-period strain and b) fibre diameter strain in the AF; c) scatter plot of D-period strain vs. initial D-period in the anterior (blue) and posterior (orange) regions.

Results

A bulk IVD compression of 2 % resulted in a reduction in the minimum distance between the vertebral endplates of 5.2 %, a tissue level compressive strain of 4.4 ± 3.2 % (Fig. 1a), fibre strain magnitude of 2.7 ± 2.9 % (Fig. 1c), and fibrillar strain magnitude of 0.018 ± 0.015 % (Fig. 2a). This hierarchical strain pattern likely arises due to load sharing between fibrous and matrix components, with composition varying across the IVD. At the tissue level, both lamellar and trans lamellar strain patterns were observed (Fig. 1b). Tissue level compression was greater in the posterior (5.7 ± 3.4 %) than the anterior (3.7 ± 2.8 %) AF (Fig. 1a). At the fibre level, strain showed region specific correlations with fibre curvature – showing negative correlation in the posterior ($p < 0.01$) and positive correlation in the anterior region ($p < 0.01$).

Correlation of values for fibres before and after loading provided estimates of per-fibre strain in nanoscale properties: approx. -0.1–0.1% D-period strain (Fig. 2a); -1–3 % D-period variation strain; -1–10% fibre diameter strain (Fig. 2b). At the nanoscale fibrillar level, prestrain (initial D-period) was found to play a key role, showing a significant negative correlation with D-period strain ($r^2 = 0.68$, $p < 0.01$, Fig 2c). Fibrils under prestrain (tension or compression) experienced greater fibril level strain, but reduced fibre level strain, with fibres under an initial tensile prestrain undergoing compression and vice versa.

Discussion

This study has revealed new insights into the hierarchical biomechanics of the IVD, uncovering a complex mechanical interplay across spatial scales with distinct regional variation. The nanoscale results highlight the role of prestrain in fibrous tissue mechanics, and this multiscale experimental data can be used to develop and validate multiscale computational models. The TomoSAXS method presented here will provide new insights into fibrous tissue biomechanics and has many potential future applications.

Acknowledgements

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