CYCLIC LOADING OF HEALTHY AND DEGRADED CARTILAGE AND THE 3D COLLAGEN FIBRILLAR RESPONSE

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Introduction

The collagen fibrillar network in articular cartilage (AC) plays a crucial role in providing the tissue with its structural integrity alongside aiding the biomechanical response of the tissue and thus enabling normal joint function [1]. To date, there is limited knowledge into the structural response of the Type II collagen fibrils in response to repetitive cyclic loading. Here, we apply synchrotron small-angle X-ray scattering (SAXS) combined with *in-situ* cyclic loading of bovine articular cartilage explants [2,3]. With a focus on the deep zone fibrils, we investigate the changes to the network in terms of orientation, fibrillar strain and inter-fibrillar variability in cartilage with and without the treatment of the pro-inflammatory cytokine IL-1β [3]. We investigate the 3-dimensional orientation response inferred by a 3D reconstruction of X-ray scattering peak intensity distributions from the 2D patterns.

Materials and Methods

Full thickness cartilage explants from the metacarpal-phalangeal joint of bovine steers (18-24 months) were excised with 2 mm biopsy punches. The explants were incubated for 12 days in serum-free supplemented DMEM with and without IL-1β (5 ng/ml, Peprotech, UK). Following treatment, samples were tested at the SAXS beamline ID02 at The European Synchrotron Radiation Facility (ESRF, Grenoble, France). Using a custom-built micro-compression tester (Fig. 1A and [2,3]), samples were subjected to 150 cycles at 0.25Hz at 20% strain. SAXS patterns were acquired within the deep zone for every 5th cycle at both 0% and 20% strain levels. Resultant patterns were analyzed for change in total peak intensity (related to intrafibrillar order), fibril D-period (pre-strain) with associated inter-fibrillar pre-strain variability (w_q), and fibril orientation.

Results and Discussion

We show that under cyclic loading there is a reversible increase in the fibrillar orientation distribution width whilst a largely constant direction of orientation is maintained. Through 3D reconstruction of the X-ray scattering peak intensity distributions, we show that the effect on the fibrillar network is a 3-dimensional conical orientation broadening around the normal to the joint surface. Further, at the intrafibrillar level, this effect is coupled with reversible reduction in fibrillar pre-strain under compression, alongside increase in the variability of fibrillar pre-strain. In IL-1 βdegraded cartilage, the collagen rearrangement under cyclic loading is disrupted and associated with reduced tissue stiffness. These finding have implications as to how changes in local collagen nanomechanics might drive disease progression and how these may link to ageing and osteoarthritis progression and thus, provides a pathway to a mechanistic understanding of such diseases.

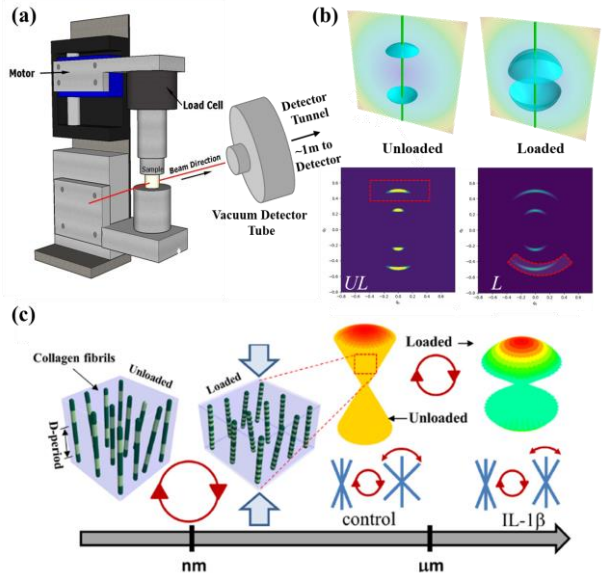

Figure 1: (A) Experimental setup: microcompression tester in SAXS beamline (B) 3D SAXS model simulations of collagen fibrils in axially symmetric narrow (left) and broad (right) orientation distributions with the associated schematic 2D SAXS patterns represented below (C) model of the nano- and microstructural dynamics of cartilage collagen fibrils under cyclic compression indicating the reduction in fibril pre-strain (left) alongside a broadening of the fibril orientation distribution (right), with a reduced orientation change under IL-1β treatment.

References

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