Multi-photon microscopy of cartilage

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Abstract

Articular cartilage has been imaged using the following multi-photon modalities: Second Harmonic Generation (SHG), Two-photon Fluorescence (TPF) and Coherent Anti-Stokes Raman Scattering (CARS). A simple epi detection microscope was constructed for SHG and TPF imaging in the early stages of this research. Later the imaging was transferred to a new microscope system which allowed simultaneous forwards and epi detection and combined CARS imaging with TPF and SHG.

Multiphoton spectroscopic studies were conducted on both intact tissue samples and the major components of the extracellular matrix, in order to identify sources of TPF. Fluorescence was detected from type II collagen, elastin and samples of purified collagen and elastin crosslinks. Age related glycation crosslinks of collagen may be a significant source of TPF. No fluorescence was detected from proteoglycans.

In intact, unfixed healthy articular cartilage the cells were observed via CARS, surrounded in their pericellular matrix which is characterised by an increase in TPF. The collagen of the extra cellular matrix showed up clearly in the SHG images. Diseased cartilage was also imaged revealing microscopic lesion at the articular surface in early osteoarthritis and highly fibrous collagen structures and cell clusters in more advanced degeneration.

In young healthy cartilage a network of elastin fibres were found lying parallel to the articular surface in the most superficial 50μm of the tissue. Regional variations in these fibres were also investigated. The fibres appeared mainly long and straight suggesting that they may be under tension, further work is needed to identify whether they have a mechanical function.

The polarization sensitivity of the SHG from collagen has been investigated for both cartilage and tendon. In the most superficial tissue these measurements can be used directly to determine the collagen fibre orientation. However at increasing depths the effects of biattenuation and birefringence must be considered. Healthy cartilage has a characteristic

pattern of polarization sensitivity with depth and this changes at lesions indicating a disruption of the normal collagen architecture.

The methods developed in this thesis demonstrate the use of non-linear microscopy to visualise the structure of the extracellular matrix and cells in intact unstained tissue. They should also be appropriate in many areas of cell and matrix biology.

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