

Micro Observations the Intervertebral Disc

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INTRODUCTION: The Intervertebral discs (IVD) function and dysfunction is governed by the structural architecture of collagen fibres in the annulus fibrosus (AF); at the macro scale for the overall performance of the disc and at the micro scale where it influences cell behaviour. To understand such mechanical behaviour the three-dimensional collagen fibre architecture must be quantified in intact IVDs. Conventional imaging modalities lack either the spatial resolution (e.g. x-ray diffraction [1]) or have limited penetration (e.g. optical, electron and confocal laser microscopy [2]) to yield mechanically important information. This study aims to validate the origin of the alternating layers of fibre texture observed within intact, hydrated and unfixed IVDs using Scanning Acoustic Microscopy (SAM) [3].

METHODS: The three-dimensional structure of intact, unfixed human lumbar IVDs was imaged using SAM at 50MHz in pulse-echo mode. The micro-structure within the AF was observed and compared to optical and electron micrographs of individual lamellae, peeled by micro-dissection.

RESULTS: SAM images of the sagittal section of the disc is characterised by alternate light and darks bands (see Figure 1a) representing the interface between adjacent lamellae and collagen bundles respectively. Within the dark bands a uniform, highly oriented fibre texture that reversed between adjacent layers was observed (shown in Figure 1b). Resolution of the texture was limited by the acoustic system to 30 μm .

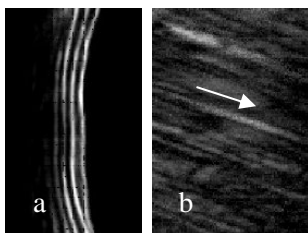


Figure 1

Optical microscopy (Figure 2) revealed that each lamella consisted of a highly organised

fibrous structure with regularly spaced splits between fibres (spacing of $25 \pm 5 \mu\text{m}$).

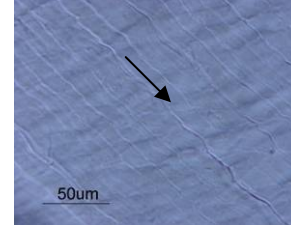


Figure 2

Electron microscopy demonstrated highly orientated collagen fibrils (diameter of 200 nm) identified by a banding structure (periodicity 60 nm) (see Figure 3b). Groups of collagen fibres of diameter 5 μm were observed (shown in Figure 3a).

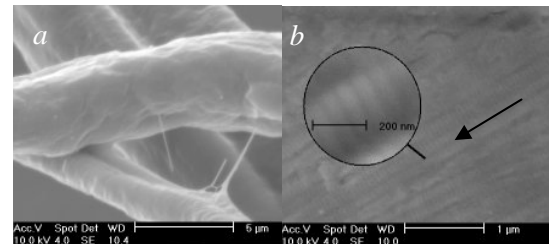


Figure 3

DISCUSSION & CONCLUSIONS: SAM operating at 50 MHz cannot resolve and therefore image individual collagen fibres on the micro scale. However, the regular defects in the fibre layers can be visualised as alternating layers of fibre texture and therefore provides an effective way of quantifying the three-dimensional fibrous structure of intact, hydrated, unfixed intervertebral discs.

REFERENCES:

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