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Characterisation of Intervertebral Discs using MID-IR Spectroscopic Imaging

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Introduction

Lower back pain affects millions of people worldwide, and has been linked to degenerative changes in the intervertebral disc (IVD) of the spine. In the 'NPmimetic' project, a multidisciplinary team has come together to create a biomimetic nano-polymer based implant and develop a minimally invasive therapy to reconstruct and regenerate diseased nucleus pulposus (NP) (http://npmimetic.com/). For a successful regeneration, tissue integrity together with the right mechanical environment is essential for normal cell function. In order to develop suitable biomimetic implants a thorough characterisation profile, that can be used as an aspirational target is important. In this study we use FTIR microscopic imaging to obtain chemical maps of control and *in vivo* CABC degenerated goat IVDs [1, 2].

Materials and Methods

Goat intervertebral discs were kindly provided from VU University Medical Centre (VUmc), Amsterdam. The intervertebral discs were formalin fixed (10%, overnight), paraffin embedded and 4 µm sections were mounted on custom made reflective steel slides. Fourier transform infrared (FTIR) microscopy in transflection-mode was used to generate molecular distribution maps from unstained paraffin sections. In this study an area of 23.45 x 28.00 mm (80 x 64 IR images) was measured resulting in a total of 10720 IR spectra per IVD section. FTIR mosaic imaging generally generates many thousands of data points. A major challenge is handling and analysing such large and chemically complex data sets in order to extract meaningful information. However, using iterative multivariate curve resolution (MCR) techniques on the reduced data matrix from principal component analysis (PCA) of 2nd derivative spectra it is possible to deconvolute highly overlapping infrared peaks into single contributions of different molecular species.

Results

The chemical identity of the extracted component using an iterative MCR algorithm is determined by comparing the extracted spectral profiles with the spectral profiles of reference materials for proteoglycan and collagen. Characteristic features matching typical proteoglycan and collagen spectra are observed. The distribution of the extracted MCR factors for collagen and proteoglycan are compared to consecutive IVD sections which were stained using traditional histological methods; Masson trichrome staining for collagen and Alcian blue staining for proteoglycan. A good match can be observed between the distribution maps of collagen and proteoglycans derived from FTIR microscopic imaging measurements and the traditionally stained sections.

Discussion and Conclusions

FTIR microscopic imaging in transflection-mode has been successfully used to generate molecular maps of collagen and proteoglycan of control and degenerate IVD sections without the use of contrast-enhancing agents. The use of 2nd derivative spectra together with the application of iterative MCR algorithms shows great potential to enhance the chemical specificity of FTIR mosaic imaging and opens up the possibility to distinguish between different proteoglycans and proteins as well as their secondary structure.

References

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Disclosures

Authors have nothing to disclose.