Characterization Of Intervertebral Disc Aging And Degeneration Using FTIR Spectroscopy

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Disclosures:

Introduction: Degenerative disc disease treatment is shifting toward the prevention and treatment of underlying etiologic processes at the level of the disc matrix composition, and integrity of the disc. The ability to perform such treatment relies on the ability to accurately and objectively assess the state of the matrix and the effectiveness of treatment. It is important not only to understand the mechanisms involved in the healing process but also to monitor and evaluate the quality of the repaired tissue. Unfortunately, the currently applied biochemical methods are not ideal for taking into account the biochemical composition as a whole. Therefore, different compositional parameters usually are individually correlated with biomechanical parameters and specific interactions between the macromolecules are not clear. Fourier transform infrared (FTIR) spectroscopy is a technique which is able to detect subtle changes in the concentration and spatial distribution of the tissue components. FTIR is typically conducted on thin (<10 mm) tissue sections, where broadband infrared absorption is measured point-by-point from the sample. Once the spectral maps are calculated, the spatial quantitative and qualitative information on the composition and organization of the tissue compounds could be obtained. FTIR has been successfully utilized in bone and cartilage research and in the differentiation between normal and diseased tissues (collagen, proteoglycans, hydroxyapatite, cross-links). The objective of this study was to establish a novel methodology to characterize the composition and microstructural spatial organization of the IVD tissue during degeneration and repair.

Methods: Sample preparation: The first two IVDs of bovine tails of four different ages (12, 18, 24, 30 months) were separated from their adjacent vertebral bodies by removing the bone and retaining the cartilaginous endplates. All tissues were fixed in Accustain (Sigma-Aldrich, St Louis, MO), paraffin embedded and sectioned into 4-mm-thick sagittal sections. Consecutive slices were assessed for histological assessment and FTIR procedure. Human IVDs were obtained from donor lumbar spines of Thompson grade 2 to 5 through organ donations within 24 hrs after death. Two IVDs per grade of degeneration were used for histological assessment and FTIR procedure. All tissues were fixed in Accustain (Sigma-Aldrich, St Louis, MO), paraffin embedded and sectioned into 4-mm-thick sagittal sections. Consecutive slices were assessed for chemical and histological properties. FTIR spectra acquisition: Bovine/human IVD sections were placed on BaF2 infrared transparent windows and infrared absorbance spectra were acquired using the FTIR system composed of a classical FTIR spectrometer FTS 7000series’ DGILAB’ coupled to UMA 600 microscope. For FTIR spectra acquisition, the system was used in point mode (aperture of 250 x250 µm) with a 4.0 cm-1 resolution and using 128 scans in transmittance mode, and spectra acquisitions were performed under complete N2 purge of the analytical system. Four repeated scans were performed on the spectral region of on the spectral region of ~900 to 2000 cm-1 in each sample in the annulus fibrosus (AF) and nucleus pulposus (NP) regions. Analysis of FTIR absorption spectrum of IVD: Spectral data were first analyzed using a univariate analysis method aimed to calculate the collagen (COL), elastin and proteoglycan (PG) content, followed by a multivariate analysis, to evaluate the collagen maturity.

Results: A clear qualitative difference was observed with aging and degeneration in the bovine and human IVDs spectra. In AF, a significant decrease of COL, elastin and PGs content, was detected accordingly to the bovine age. This decrease was also associated with an increase in collagen maturity. In human IVDs, both AF and NP showed similar results as bovine samples. The degeneration was associated with a decrease in COL, elastin and PGs content and an increase in COL maturity.

Discussion: Our results gave molecular bases for a functional investigation of IVDs in normal and degenerative conditions. The combined use of the submolecular parameters which appeared the most discriminant should allow a strong improvement of the identification capacity of the ECM components composing IVD by FTIR spectroscopic imaging.

Significance: FTIR spectroscopy and its imaging-related technique provides an innovative method in the field of molecular histology, offering an aid in the diagnosis of a wide range of IVD pathologies.
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