Combined AFM micro-indentation and MALDI MSI for correlation of mechanics and composition of menisci

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INTRODUCTION: Meniscus degeneration leads to a change in load distribution and a loss of cartilage volume. These two factors may cause chronic pain and lead to knee osteoarthritis. For better clinical treatment the physico-chemical hallmarks of meniscus degeneration need further elucidation. Meniscal tissue mainly consists of collagen (75% of dry weight). Collagen types I and II are the most abundant and form fibrils (supramolecular structures), which largely contribute to the tissue mechanical properties. Given the small number of existing studies, knowledge of meniscal tissue micro-mechanical properties and corresponding molecular composition is scarce. In this first study, we present a proof of principle for multimodal physico-chemical assessment. The mechanical properties and correlated chemical composition of the same meniscal tissue sections were assessed via atomic-force microscopy (AFM) micro-indentation and matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI), respectively. We further hypothesize that there is a correlation between indentation modulus and proteomic composition.

METHODS: Human meniscus samples (sourced from donors by the Anatomy Department, Medical University of Vienna and approved by the local IRB) were cut into 10 µm thick cryosections and deposited on Poly-L-Lysin-coated microscopic slides. AFM micro-indentation was conducted (Nanowizard Ultraspeed A, JPK-Bruker) using a cantilever (NP-O10-D, 0.1N/m spring const., freq.18 kHz) equipped with a 10 µm diameter borosilicate glass spherical tip (1.0 nN setpoint) in physiological conditions (Ringer acetate buffer pH = 7,4 / Roche cOmplete, Mini, EDTA-free Protease Inhibitor Tablets – 1 tablet per 10 ml). Force maps 450×450 µm at 50 µm spatial resolution were measured in ROIs, red-red (outer), red-white (middle), and white-white zones (inner) (Fig. 1), and force curves were analyzed with the Hertz model [1]. Before on-tissue digestion lipids and salts were removed by sequential washing in ethanol, Carnoy's buffer, and water. Samples were sprayed with Trypsin/Lys-C, collagenase III by HTX TM-Sprayer™ (HTX Technologies LLC) and incubated for 3-4 hours at 37° C. After incubation, α-cyano-4hydroxycinnamic acid (CHCA) was also applied via spraying. Samples were measured at MALDI-TOF/TOF and 7T FTICR system (ultrafleXtreme/scimaX, Bruker) at 30 µm to 100 µm lateral resolution, and mass-spectra in the range 700–3500 m/z were obtained. Images with the distribution of peptides were co-registered by flexImaging and SCiLS (Bruker). To evaluate the collagen content, images were processed in ImageJ. The Kruskal-Wallis test was used to compare the indentation moduli of three regions, p-value < 0.05 was considered statistically significant.

RESULTS: Micro-indentation tests revealed different properties for the different meniscus regions; the red-red, red-white, and white-white zones were significantly different from each other ($p < 0.001$, Fig. 1). In obtained mass-spectra signals from various types of collagens were found (e.g., collagen I–VI, X, and XVII). Our MSI results show that collagen type I, the main structural component of the meniscus sample, is unevenly distributed over all regions. (Fig. 2a). Some types of collagen are distributed only in certain parts, e.g., COL2A1 is predominantly located on the edges of the meniscus section (Fig. 2a, pink) and COL17A1 was measured in the superficial layer (Fig. 2b, blue).

DISCUSSION: In this study, we demonstrate a multimodal imaging approach using AFM and MALDI MSI conducted on the same samples. We show that the spatial distribution of different collagen types is correlated with the indentation modulus. The amount of collagen type I and III (associated with I) is lower in the white-white zone, which shows the lowest indentation modulus, compared to the red-red and red-white zones. Meanwhile, the highest content of collagen XVII was detected in the white-white zone (Fig. 3, blue). Although, the red-red region has the highest indentation modulus, the content of collagens I and III is lower than in the red-white zone (Fig. 3, yellow and orange). The amount of collagen II is roughly the same in all measured regions (Fig. 3, pink). Our results of indentation moduli are in agreement with earlier studies [2, 3], where the lowest indentation modulus was obtained for the inner region in comparison to others (middle and outer).

SIGNIFICANCE/CLINICAL RELEVANCE: The multimodal combination of methods for tissue imaging allows correlative assessment of the physico-chemical properties of tissue sections in a spatially-resolved manner. We expect this to aid in identifying hallmarks of meniscal degeneration at the µm-scale.

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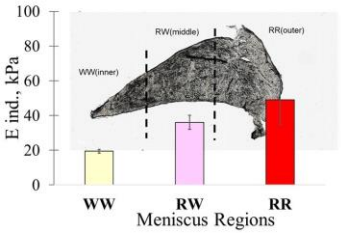

Fig. 1 Results for indentation modulus of human meniscus in different regions ($p < 0.001$, for all comparisons).

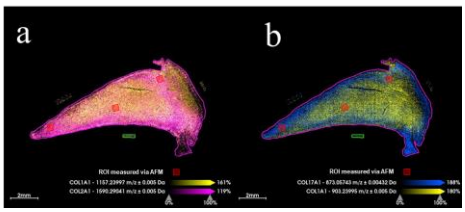

Fig. 2 Representative images and corresponding m/z values of four collagen peptides: a – COL1A1 (yellow) and COL2A1 (pink); b - COL1A1 (yellow) and COL17A1 (blue).

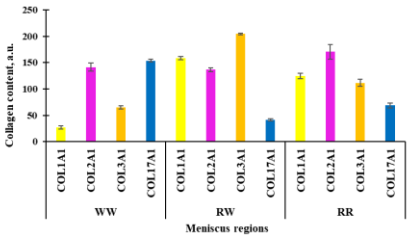

Fig. 3 Distribution of collagen type I (yellow), II (pink), III (orange) and XVII (blue) among meniscus regions.