SUPPLEMENTARY MATERIAL

In the following, the used microscopic and spectroscopic methods are presented in detail.

1.1.3. SAMPLE PROCESSING

The samples fixed in formalin were decalcified and dehydrated in an increasing series of alcohol solutions, after which they were embedded in paraffin (Paraplast Plus, Lancer Division of Sherwood medical, Kildare, Ireland). Histological sections were then cut with a microtome (LKB 2218 HistoRange microtome, LKB produkter Ab, Bromma, Sweden) perpendicular to the cartilage surface. Section thicknesses were ~3 μm for DD and ~5 μm for PLM and FTIRI. DD sections were stained with safranin O[1] and for PLM and FTIRI sections, PGs were enzymatically removed prior to the measurements.

FOURIER TRANSFORM INFRARED SPECTROSCOPIC IMAGING

Fourier Transform Infrared Imaging (FTIRI) was used to determine the spatial distribution of collagen content[2-5]. A Perkin Elmer Spotlight 300 (Perkin Elmer, Shelton, CO, USA), which had CO_2-free dry air purge system (FT-IR purge gas generator, Parker Hannifin corporation, Haverhill, MA, USA) was utilized. Three sections were measured from each sample. Produced sections were installed on BaF_2 windows. FTIRI data from the sections was collected using 4 cm^{-1} spectral resolution and 6.25 μm pixel resolution with 4 repeated scans. Collagen content was estimated by integration of the amide I region (1585 – 1720 cm^{-1}) (Fig. 2). The measured pixel values were averaged in the

transversal direction and the average depth-wise collagen profiles were calculated from the three sections from each sample (Fig. 2). Analyses of the IR spectra were done using custom-made Matlab scripts.

1.1.4. POLARIZED LIGHT MICROSCOPY

PLM was used to analyze the collagen orientation angles of the samples in a depth-dependent manner[6,7]. In PLM, the collagen fibers parallel to the cartilage surface give an angle of 0° (superficial zone) and the fibers perpendicular to the cartilage surface give an angle of 90° (deep zone). Measurements were done with Leitz Ortholux II POL -polarized microscope (Leitz, Wetzlar, Germany) with 4x magnification, equipped with CCD camera (Photometrics CH 250/A, Photometrics Inc., Tucson, AZ, USA). The measured pixel values were averaged in the transversal direction and the average depth-wise birefringence profiles were calculated from three sections (Fig. 3). Calculation of the collagen orientation of the samples was based on Stokes parameters[6,7].

1.1.5. DIGITAL DENSITOMETRY AND MANKIN SCORING

Spatial distribution of PGs was determined using the digital densitometry of Safranin O stained histological sections[8,9]. Sections were measured with a computer-controlled CCD camera (SenSys, Photometrics Inc., Tucson, AZ, USA) mounted on a light microscope (Leitz Orthoplan, Leitz, Wetzlar, Germany). Grayscale images of the Safranin O stained sections were captured and the optical density was used to estimate the tissue fixed charge density and distribution of PGs. The pixel values were averaged in the transversal

direction and the average depth-wise profiles were created from three sections of each sample (Fig. 4). After DD, the sections were used to determine the Mankin score of the samples, which reflects the severity of OA of the cartilage samples[10, 11].

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