INTRODUCTION:
Extracellular matrix (ECM) of articular cartilage consists mainly of fibrillar collagen (type II) and proteoglycans (PGs). Composition and properties of ECM generate unique biomechanical properties of articular cartilage. ECM composition varies in different layers of the tissue. Collagen content is high in the superficial and deep layers of the tissue whereas PGs show nearly monotonously increasing concentration from superficial to deep tissue. Solid matrix composition directly determines water distribution within tissue. Detailed description of the structural characteristics of the matrix is essential in order to understand complex mechanical features of articular cartilage. As the traditional biochemical methods fail to preserve spatial compositional information, these techniques are often limited to the determination to average (bulk) content information of collagen or PGs only. Therefore, compositional differences of territorial and interterritorial ECM are not well characterized. The present study introduces Fourier transform infrared imaging spectroscopy (FT-IRIS) for the characterization of ECM composition, revealing detailed compositional differences between the territorial and interterritorial matrix.

METHODS
Cartilage samples were prepared from femur, tibia and patellar groove of human cadaver knee joints (n=13). Full-thickness cartilage layer was detached from the subchondral bone with a razor blade. Cartilage was embedded into OCT embedding media with two pieces of nitrocellulose membrane. Nitrocellulose was used to normalize differences in section thickness as previously described [1]. Subsequently, 10 µm-thick sections were cut with cryomicrotome and sections were directly placed onto ZnSe-windows. After air-drying (minimum 24 h) samples were measured using PerkinElmer Spectrum Spotlight 300-imaging system. IR-images were acquired with 8 cm⁻¹ spectral and 6.25 µm-pixel resolution and two repeated scans were averaged for each pixel. Collagen content was analyzed using the average background corrected absorption of amide region (1710-1510 cm⁻¹) and PGs with carbon region absorption (1075-975 cm⁻¹).

RESULTS
FT-IRIS measurement showed high collagen contents in the superficial and deep cartilage whereas PG concentration was monotonically increasing (Fig. 1). Highest collagen and PG levels were reached in the deep cartilage. Especially in the deep cartilage, PG concentration around cells increased (Fig. 1 and 2b). Territorial matrix surrounding the cell showed distinctively different composition, as compared to interterritorial matrix between the cells. Territorial matrix revealed higher PG concentration and lower collagen content compared to interterritorial matrix (Fig. 2a and b).

DISCUSSION
Present study demonstrated the suitability of FT-IRIS for characterization of highly inhomogeneous ECM composition. Chemical images showed complex distribution patterns of collagen and PGs around the cells. PG distribution, even its high concentration around the cells, can also be seen using light microscopy with staining techniques, whereas the methods for quantification of spatial collagen distribution have been lacking. Both collagen and PG distributions were effectively measured with a singe imaging session. This quantitative information on the main matrix constituents, and especially their distribution around the cells, allows us to develop more realistic theoretical models to describe mechanical interactions, the fluid flow and stresses, between the cell and its matrix environment. Detailed information on the physiological conditions at the chondrocyte-ECM interface is essential to improve understanding of mechanotransduction in cartilage.

REFERENCES

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