Relationships between cell volume, structure, composition and mechanical properties of osteoarthritic human hip articular cartilage

INTRODUCTION: Osteoarthritis (OA) is a common joint disease, which alters the structure and composition of articular cartilage, leading to tissue destruction. Reduction in the proteoglycan (PG) and collagen contents and fibrillation of the collagen fibrils are typical signs of OA. Chondrocyte volumes have also been shown to increase with OA, which may be connected to the altered biosynthetic activity of chondrocytes. However, it has not been shown how chondrocyte volumes are related to specific structural, compositional and mechanical properties of human hip articular cartilage during OA progression.

The aim of this study was to experimentally measure chondrocyte volumes, biomechanical properties, PG and collagen contents, and collagen fibril orientations of osteoarthritic articular cartilage from human hip joints.

METHODS: Osteochondral articular cartilage samples (n=16) were obtained from the femoral heads of patients undergoing total hip arthroplasty. The samples were collected with the permission from the National Agency for Medicolegal Affairs in Finland (permission 103/13/03/02/09). After sample preparation and storage in Dulbecco’s modified Eagle’s medium (DMEM) in incubator at 37°C over night, chondrocytes were imaged with confocal laser scanner microscope (CLSM). Subsequently, biomechanical testing of the samples was conducted, after which the samples were prepared for microscopic and spectroscopic analysis. Finally, digital densitometry (DD), polarized light microscopy (PLM), Fourier Transform Infrared microspectroscopy (FTIR) and Mankin scoring were conducted for all the samples.

CLSM: Chondrocytes were stained with both calcein-AM and propidium iodide for live and dead cells, respectively. Semi-motorized DM RXA2 upright CLSM (Leica TCS SP 2, Leica Microsystems CMS GmbH, Mannheim, Germany) was used for cell imaging. Stacks of images were obtained at a 0.2 µm vertical z-axis spacing using an objective with x63 –magnification. The pixel size in x-y plane was 0.4 µm x 0.4 µm. Chondrocytes were imaged in isotonic medium (~290 mOsM) at 37°C. The Visualization Toolkit 5.2.0 (Kitware Inc.) was used to reconstruct 3D-images of the cells and a code programmed with Python was used to calculate cell volumes.

Mechanical testing: Stepwise stress-relaxation indentation tests (4 x 5% steps, 100% loading rate, 900s relaxation time) were conducted to determine dynamic (initial E0 and strain dependent Eeq) and equilibrium (Eeq) elastic moduli of the samples.

DD, FTIR and Mankin scoring: DD measurements were conducted to determine the PG content from safranin O stained sections of the samples. Also Mankin scoring from the Safranin O stained sections was conducted. PLM was used to measure the collagen orientation of the samples from unstained sections. FTIR was used to determine the collagen content of osteoarthritic (Mankin score: 2-6) human cartilage samples from the hip joints (n=16).

RESULTS: Statistically significant positive correlation (r = 0.533, p = 0.03) between the equilibrium elastic modulus (Eeq) and the PG content of cartilage was observed (Figure 1).

The average volume of chondrocytes was 635 ± 278 µm³ and it varied from 138 to 1612 µm³, while Mankin score of samples varied from 2 to 6. Using linear correlation analysis, when the cell volume increased from the minimum to the maximum value (+1066%), the PG and collagen contents, and equilibrium and dynamic elastic moduli decreased by 13-125%, whereas the collagen orientation increased by 155% (Table 1).

DISCUSSION: We studied the relationships between cell volume, structure, composition, and function of human hip articular cartilage with various OA grades. The results indicated that along with the increase in cell volume, the PG and collagen contents and biomechanical properties of the samples were reduced, while simultaneously the fibril orientation was increased.

Chondrocyte volumes have been reported to increase in OA. Our results support this finding. However, none of the earlier studies have shown quantitative experimental results of the reasons behind this phenomenon. There are also no earlier studies of volumes of human femoral head chondrocytes in different stages of OA. Our results suggest that the collagen fibril orientation is the dominant structural parameter to modulate cell volumes in hip articular cartilage during OA progression, while the collagen and PG contents have less influence on chondrocytes. It may be that primarily the collagen fibrillation has reduced stresses and confining effects around cells, allowing cells to expand.

SIGNIFICANCE: We showed for the first time quantitative results of the relationships between cell volume and structure/function of human hip osteoarthritic cartilage. This information improves the knowledge of the cell-tissue interactions in OA, and could be applied in the future for cell volume based OA diagnostics as well as development and analysis of tissue engineered articular cartilage repair.

ACKNOWLEDGEMENTS: Academy of Finland and Sigrid Juselius Foundation.