Collagen content in the Cartilage Matrix Controls Chondrocyte Deformation in Mechanically Loaded Healthy Tissue

Ari P. Ronkainen[1], James M. Fick[1], Walter Herzog[2], Rami K. Korhonen[1]
[1]University of Eastern Finland, Kuopio, Finland, [2]Univerisity of Calgary, Calgary, AB, Canada

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Introduction: Understanding chondrocyte (i.e., cells in articular cartilage) responses is important because cellular biosynthesis is driven by the strains and stresses that chondrocytes experience [1]. Deformation behavior of chondrocytes depends on articular cartilage structure and composition. In particular, changes in collagen orientation and proteoglycan (PG) content modulate cell deformation in osteoarthritis [2]. However, this behavior has not been shown experimentally for healthy tissue. Our objective was to measure structural parameters of cartilage from different sites in the rabbit knee and to investigate which components control chondrocyte deformation under mechanical indentation loading. We hypothesized that collagen orientation and proteoglycan content play a major role in chondrocyte deformation. To test this hypothesis, collagen orientation, collagen content and proteoglycan content of rabbit knee cartilage samples were examined by microscopic and microspectroscopic methods. Chondrocyte deformations were measured previously utilizing confocal laser scanning microscopy (CLSM) [3].

Methods: Five skeletally mature female New Zealand White rabbits (13 ± 1 month) were sacrificed and their knee joint tissues were harvested [3]. All procedures were performed according to the guidelines of the Canadian Council on Animal Care, and were approved by the committee on Animal Ethics at the University of Calgary. Chondrocyte deformations were measured at the University of Calgary, using CLSM equipped with a custom indentation (diameter 2 mm) system that allowed for *in situ* measurements of superficial zone chondrocytes (figure 1A). Both knees were used and six different locations per knee joint were examined, including the patella, femoral groove, lateral and medial femoral condyles and tibial plateaus. Three samples were excluded due to inadequate fluorescein staining, which resulted in a total sample size of N = 57 (cartilage samples) and n = 570 (cells).

Following CLSM, samples were placed in formalin and shipped to the University of Eastern Finland, Kuopio, where cartilage composition and structure were analyzed (N = 57). Following sample processing, collagen content, collagen fiber orientation and proteoglycan content were quantified using Fourier transform infrared spectroscopy (FTIR), polarized light microscopy (PLM) and digital densitometry (DD), respectively [4]. For each method, a depthwise profile was acquired from a region of interest extending from the cartilage surface to the cartilage-bone interface (figure 1B).

In order to analyze the relationships between cells and tissue structure, cell deformations (cell width, depth, height and volume changes, see figure 1A) were selected as dependent variables and multilinear regression models using collagen orientation, collagen content and PG content values as independent variables were constructed. Correlation analyses were performed across selected tissue depths (0-100 µm) based on the structural analysis. All statistical analyses were conducted using SPSS 21 (IBM Corp, Armonk, NY, USA). The level of significance was set at $p < 0.05$.

Results: The best correlations between cell deformation responses and cartilage structure were found around a depth of 22 µm (figure 1C). Because cells have finite dimensions (i.e., a height of ~5-10 µm), the compositional parameters used in the rest of the correlation analyses (table 1) were calculated as the average from 15 to 30 µm in depth from the cartilage surface to capture the composition of the local matrix surrounding the cells. Collagen content was the most significant variable explaining cellular deformation, being particularly related to cell width ($p = 0.001$) and height ($p < 0.001$) changes. Collagen orientation angle contributed mostly to cell width changes ($p = 0.001$), but not to cell depth and volume changes ($p < 0.05$). PG content had a significant contribution only to the cellular volume changes ($p = 0.025$).

Discussions: Earlier studies [2] investigating cell response in osteoarthritic tissues suggested that collagen orientation and PG content are the most important structural parameters modulating chondrocyte deformations. Therefore, we hypothesized this to be the case for normal tissue as well. However, our results contradicted this hypothesis in part. Our results suggest that collagen content, which is typically not altered in early osteoarthritis [2,4], primarily controls cell responses in normal, 'healthy' tissue, and may try to maintain normal cell function also in degenerated tissue where collagen orientation and PG content are altered. In a previous theoretical work, similar cellular changes as reported here were predicted in case of collagen fibrillation (higher collagen orientation angle) or for increased ECM PG content [5]. Also, in an experimental work it was observed that *in vitro* glycation of articular cartilage, which increases collagen cross-linking, caused cellular strains to be lower in all directions, which was also observed here for increasing collagen contents (note in table 1 that cell height and volume are reduced in normal cell compression) [6].

Significance: We found that local collagen content is an important factor controlling cell deformation when normal cartilage is mechanically compressed. These cell-matrix relationships are crucial for understanding 'healthy' tissue function and should be taken into account when designing tissue engineered cartilage.

References: [1] Goldring and Marcu, Arthritis Research & therapy 11, 2009. [2] Turunen *et al*. Osteoarthritis and Cartilage 21, 2013. [3] Fick *et al*. ORS 2014 Annual Meeting, Poster: 0191. [4] Mäkelä *et al*. Osteoarthritis and Cartilage 22, 2014. [5] Tanska *et al*. Computational and Mathematical Methods in Medicine, 2013. [6] Fick *et al*. Osteoarthritis and Cartilage 22, 2014.

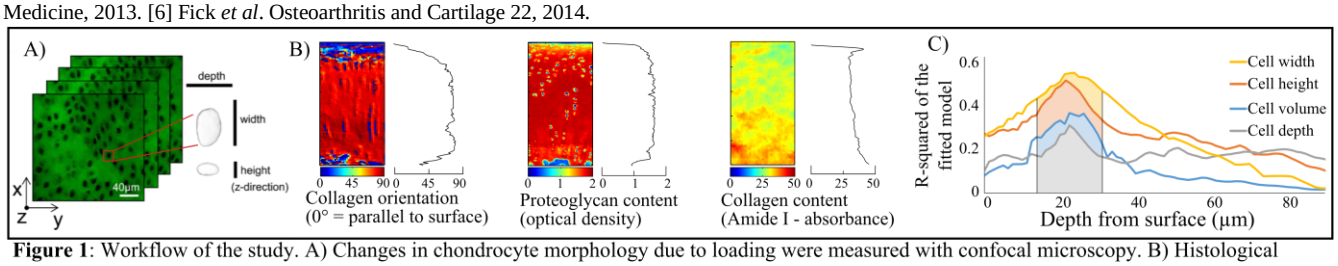

Figure 1: Workflow of the study. A) Changes in chondrocyte morphology due to loading were measured with confocal microscopy. B) Histological samples were imaged with three different methods (PLM, DD, FTIR) to quantify collagen orientation, proteoglycan content and collagen content. C) Multilinear regression models were built at different cartilage depths to investigate correlation between the structure and chondrocyte deformations. For the final analysis, a range from 15 to 30 µm was chosen. Note graph c) only shows first 100 µm from tissue surface.

Table 1: Summary of the linear regression models. Shown are regression coefficients for each independent variable: collagen orientation (PLM, degrees), proteoglycan content (DD, optical density) and collagen content (FTIR, absorbance), their mean ± 95% confidence intervals and their significance. Also R-squared values are presented for each model.

Dependent Variable (mean ± 95% CI)	Whole model R-squared	Regression coefficients (mean ± 95% CI)			Significance (p - value)		
		PLM (31.8 ± 1.8) °	DD (1.16 ± 0.09) optical density	FTIR (10.2 ± 1.4) absorbance	PLM	DD	FTIR
Width change (7.4 ± 1.7) %	**0.573**	**0.240**	-2.884	**-0.447**	**0.001**	ns	**0.001**
Depth change (7.1 ± 1.8) %	**0.388**	0.140	0.842	**-0.362**	ns	ns	**0.046**
Height change (-19.6 ± 2.1) %	**0.520**	**-0.285**	4.932	**0.962**	**0.049**	ns	**< 0.001**
Volume change (9.4 ± 1.8) %	**0.411**	0.003	**5.565**	**0.558**	ns	**0.025**	**0.002**