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Depth-wise progression of osteoarthritis in human articular cartilage: investigation of composition, structure and biomechanics

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Summary

Objective: Osteoarthritis (OA) is characterized by the changes in structure and composition of articular cartilage. However, it is not fully known, what is the depth-wise change in two major components of the cartilage solid matrix, i.e., collagen and proteoglycans (PGs), during OA progression. Further, it is unknown how the depth-wise changes affect local tissue strains during compression. Our aim was to address these issues.

Methods: Data from the previous microscopic and biochemical measurements of the collagen content, distribution and orientation, PG content and distribution, water content and histological grade of normal and degenerated human patellar articular cartilage ($n = 73$) were reanalyzed in a depth-wise manner. Using this information, a composition-based finite element (FE) model was used to estimate tissue function solely based on its composition and structure.

Results: The orientation angle of collagen fibrils in the superficial zone of cartilage was significantly less parallel to the surface ($P < 0.05$) in samples with early degeneration than in healthy samples. Similarly, PG content was reduced in the superficial zone in early OA ($P < 0.05$). However, collagen content decreased significantly only at the advanced stage of OA ($P < 0.05$). The composition-based FE model showed that under a constant stress, local tissue strains increased as OA progressed.

Conclusion: For the first time, depth-wise point-by-point statistical comparisons of structure and composition of human articular cartilage were conducted. The present results indicated that early OA is primarily characterized by the changes in collagen orientation and PG content in the superficial zone, while collagen content does not change until OA has progressed to its late stage. Our simulation results suggest that impact loads in OA joint could create a risk for tissue failure and cell death.

Keywords: Articular cartilage, Osteoarthritis, Collagen, Proteoglycans, Fourier transform infrared imaging, Polarization microscopy, Finite element analysis.

Introduction

Osteoarthritis (OA) is a degenerative joint disease with significant impact sociologically, economically and on well-being. Progressive degeneration of articular cartilage is mainly characteristic to the development of OA. PG depletion has been reported to occur in the early stage of OA, especially in the superficial tissue zone[1–5]. Simultaneously, alterations in the collagen fibril network have been observed including extensive changes in the collagen fibril orientations, especially in the superficial zone[3,4,6,7], and reduction in the collagen content.[4] These structural changes lead to cartilage swelling and increased water content, increasing tissue permeability and allowing free water and other molecules to flow in and out of the tissue. All of these changes together debilitate the mechanical integrity of articular cartilage by decreasing its mechanical stiffness. In this stage, a non-homeostasis may exist between the functional demand and tissue properties, which further accelerates the degeneration of the tissue.

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Despite the fact that the structural and compositional changes are known to occur in OA, many studies of articular cartilage and OA progression are based on experimental animal models or limited amount of human samples. Furthermore, OA changes are often qualitatively graded using traditional histological techniques. With regard to quantitative techniques, biochemistry offers a way to investigate the chemical composition of articular cartilage. However, the depth-wise biochemical analysis is challenging to conduct and, consequently, bulk values for the entire tissue are often reported in the literature. Thus, a comprehensive depth-wise characterization of the changes in composition and structure of human articular cartilage during the development of OA is still lacking.

Since articular cartilage has a demanding mechanical function in the joint, prediction of the mechanical performance of cartilage during joint loading would be an asset for the diagnosis and possible future treatment of OA. However, experimental determination of the short-term mechanical performance of cartilage requires invasive techniques, and the long-term mechanical behaviour is even impossible to measure clinically. Recently, a finite element (FE) model being able to estimate functional properties of articular cartilage based on tissue composition was presented[8,9]. Provided that one could differentiate between the normal and osteoarthritic cartilage

Table I
Thickness and biochemical parameters of the samples. These parameters as well as the microscopic information of the samples (Figs. 2, 3, 5) were implemented in the FE models (Fig. 6). See text and Julkunen et al.[8] *for further details of the implementation*

Group	Thickness (mm)	Collagen fraction (%)	PG fraction (%)	Water fraction (−)	FCD (mEq/ml)
Intact	2.985	79.49	20.51	0.764	0.09
Early OA	2.890	82.10	17.90	0.757	0.06
Advanced OA	2.648	84.67	15.33	0.785	0.04

function solely based on tissue composition and structure, it could be a major step forward in the clinical evaluation of articular cartilage and OA. In the clinic, the geometry, structure and composition of cartilage could be obtained for the model, e.g., from quantitative magnetic resonance imaging (MRI)[10,11]. To achieve that goal, a comprehensive microscopic characterization of the depth-wise composition and structure of articular cartilage, as well as the relationships between the microscopic, MRI and mechanical properties of the tissue should be first clarified.

The aim of the present study was to characterize depth-wise changes in the composition and structure of normal and osteoarthritic (early and advanced OA) human articular cartilage in a large sample material. Data from extensive microscopical and biochemical measurements were collected and reanalyzed. Finally, a composition-based FE model was used to estimate the local tissue strains in normal and OA cartilage under a constant contact stress.

Methods

STUDY PROTOCOL

In the present study, the experimental data from our earlier studies[8,12] was gathered and reanalyzed. In those studies, Fourier Transform Infrared imaging (FTIRI), histological analyses and biochemical measurements were conducted for human patellar cartilage samples *in vitro*. In the present study, the raw data from FTIRI measurements were re-processed and analyzed in a depth-wise manner. Furthermore, in the present study, a composition based fibril-reinforced poroviscoelastic model of articular cartilage was applied to clarify the effect of the depth-wise compositional and structural changes in OA on the mechanical behaviour of articular cartilage. In the following paragraph, the experimental study protocol of our earlier studies are briefly described, and more details can be found in the original papers[8,12].

Osteochondral samples ($n = 84$) were prepared from 14 patellae from right knees of cadaveric human donors (age 55 ± 18 years)[12]. Samples were prepared from six locations in each patella (superomedial, superolateral, central medial, central lateral, inferomedial, and inferolateral). After sample preparation, cartilage was detached from the subchondral bone and processed for microscopic and biochemical analyses. FTIRI was used to estimate spatial PG and collagen contents of the samples. Polarized light microscopy (PLM) was applied for the characterization of collagen orientation. Light microscopy was used for the histological characterization (Osteoarthritis Research Society International, OARSI grading), and biochemical

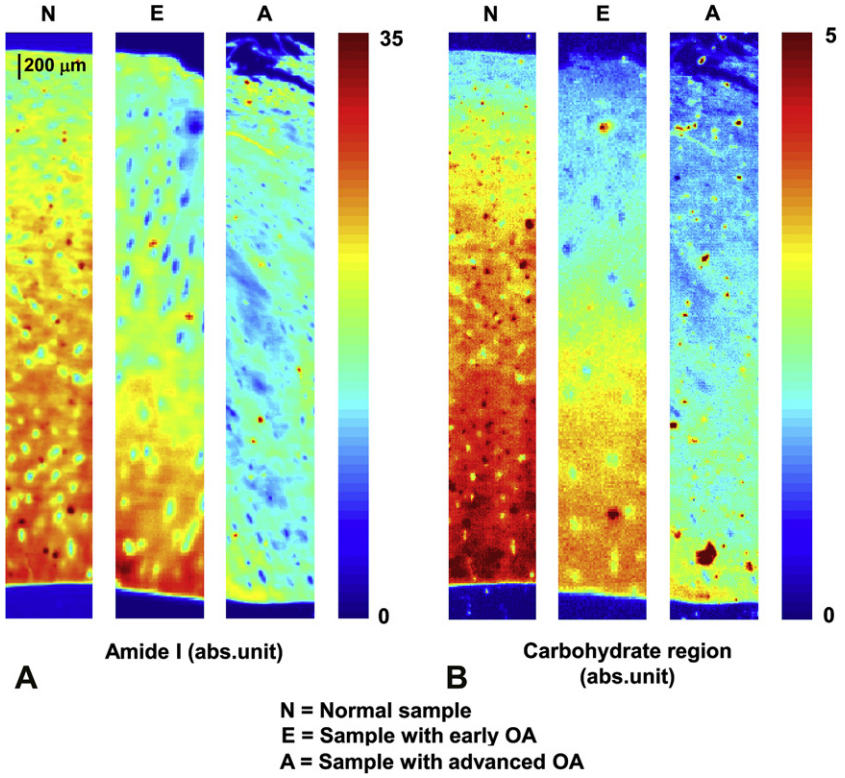

Fig. 1. Parametric images of (A) Amide I absorption, i.e., estimate of collagen content, and (B) Carbohydrate region absorption, i.e., estimate of PG content, of three representative samples (normal, early OA and advanced OA) measured with FTIRI. Spatial collagen and PG contents of the samples were estimated as the integrated absorbance of Amide I peak (1585−1720 cm^{-1}) and Carbohydrate region (1140−985 cm^{-1}), respectively, from the infrared absorption spectrum.

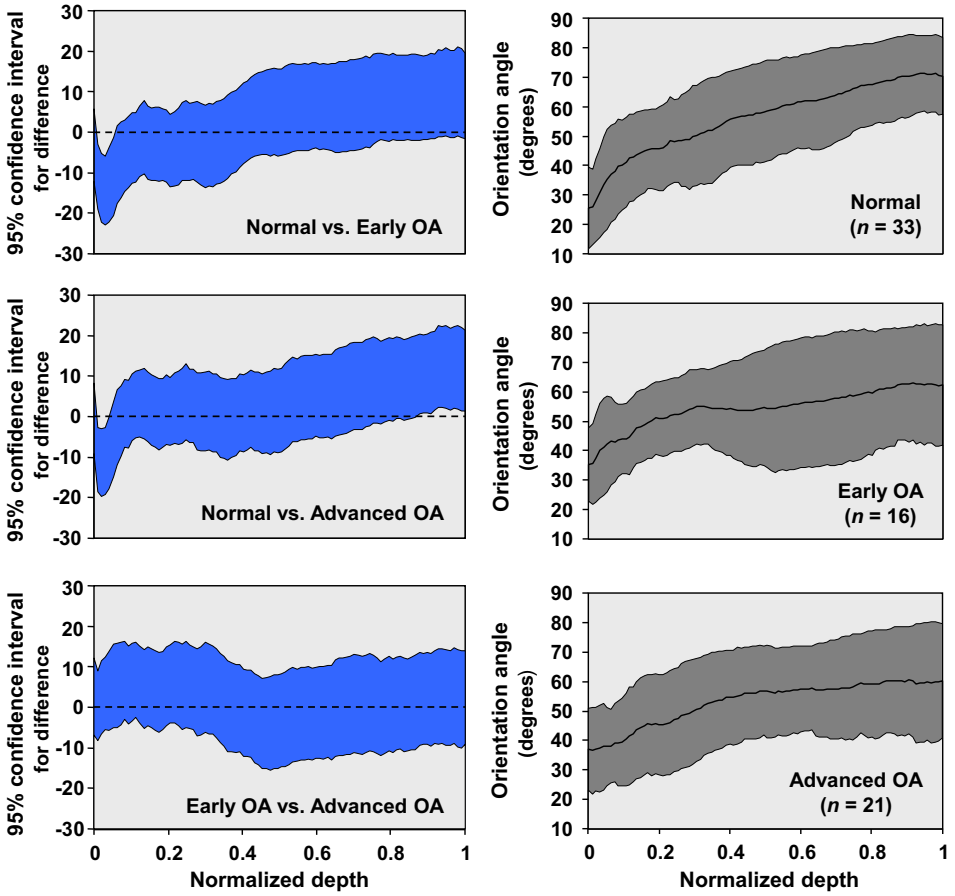

Fig. 2. Right column: Mean values (±SD) of the orientation angle in different groups (normal, early OA and advanced OA) as a function of relative tissue depth. Collagen orientation was measured with ePLM. Left column: 95% confidence intervals for the difference between estimated means of the orientation angle in different OA groups (normal vs early OA, normal vs advanced OA and early OA vs advanced OA). Mixed linear model was used in the statistical comparisons. In graphs, confidence intervals are shown as a function of relative depth of the tissue.

methods were utilized to assess collagen, PG and fluid fractions, as well as fixed charge densities (FCD) of the samples. Due to the highly advanced OA in some samples, which precluded reliable analysis, altogether 73 samples could be included in the study. Finally, information on tissue structure and composition of normal, early OA and advanced OA cartilage was implemented in the composition-based FE models, which were used to estimate local tissue strains, without mechanical testing.

HISTOLOGICAL GRADING

In our earlier study[12], histological grading was chosen as an indicator and a reference method for OA progression. OA grade of all samples was assessed according to OARSI histopathology grading system with subgrades[13]. Based on the OARSI grading, samples were divided into three groups: *normal* ($n = 35$, OARSI grade = 0), *early OA* ($n = 16$, OARSI grade = 1.0–1.5) and *advanced OA* ($n = 22$, OARSI grade = 2.0–4.5).

FTIRI

In this study, the raw data of the FTIRI measurements of the earlier study[12] were re-processed and analyzed in a depth-wise manner. For the estimation of PG and collagen contents, microscopic unstained sections (thickness = 3 μm) were prepared for FTIRI measurements. In FTIRI, infrared light absorption is measured point-by-point within the microscopic section, and an infrared absorption spectrum is determined in each pixel. Different components of biological samples show typical absorption characteristics that can be used to detect and quantify the molecule of interest, i.e., PG and collagen in the cartilage tissue. By far, FTIRI is the only experimental technique enabling simultaneous measurement of spatial distribution of PG and collagen.

Measurements were conducted using the Perkin Elmer Spectrum Spotlight 300 imaging system (Perkin Elmer, Shelton, CO, USA). Spatial pixel size was 6.25 μm, spectral resolution was set to 4 cm^{-1} wavenumber, and spectral region of 2000–670 cm^{-1} was collected. After data acquisition, a baseline correction was conducted for all spectra before further analysis. In that correction, the minimum value of the absorption spectrum in the entire spectral range was set to zero. Subsequently, spatial collagen content of the samples was estimated by measuring the integrated absorbance of the Amide I peak (1585–1720 cm^{-1}) from the infrared absorption spectrum[4,14–17]. Spatial PG content of the samples was estimated by measuring the integrated absorbance of the Carbohydrate region (1140–985 cm^{-1}) from the infrared absorption spectrum[4,14]. It has also been suggested in the literature that by normalizing the integrated absorbance of the Carbohydrate region with the absorbance of the Amide I peak, one obtains the estimate of relative PG content, which should be independent of the possible variation in thickness between microscopic sections[15–17]. In the present study, PG content was estimated with both methods, i.e., directly from the integrated area of the Carbohydrate region and using the ratio of the Carbohydrate to the Amide I.

PLM

The depth-dependent collagen fibril orientation for each sample was analyzed with the enhanced polarized light microscopy (ePLM) system (Leitz Ortholux II POL, Leitz Wetzlar, Wetzlar, Germany). First, spatial orientation map was calculated for each sample by measuring the signal intensity in each image pixel. Subsequently, image pixels were horizontally averaged to obtain depth-wise collagen orientation profiles. The analysis technique has been comprehensively described in an earlier publication[18].

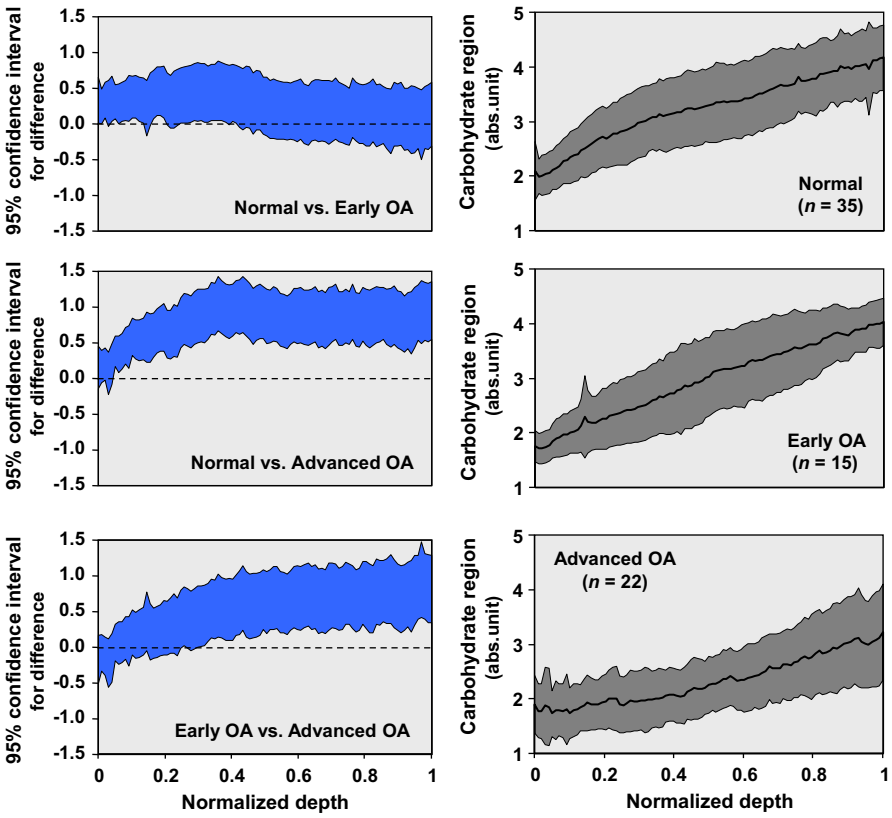

Fig. 3. Right column: Mean values (±SD) of the Carbohydrate region absorption, i.e., estimate of the PG content, in different groups (normal, early OA and advanced OA) as a function of relative tissue depth. FTIRI was used for the measurements and absorption was quantified directly from the area under the spectrum in Carbohydrate region (1140−985 cm^{-1}). Left column: 95% confidence intervals for the difference between estimated means of the Carbohydrate region absorption in different OA groups (normal vs early OA, normal vs advanced OA and early OA vs advanced OA). Mixed linear model was used in the statistical comparisons. In graphs, confidence intervals are shown as a function of relative depth of the tissue.

FE MODEL AND SIMULATIONS

In the modelling part of the study, a composition based fibril-reinforced poroviscoelastic model of articular cartilage was applied. The model is an extension of the biphasic theory[19], and it includes the compositional fractions of the solid constituents (collagen and PGs) and water, depth-dependent collagen fibril orientation, viscoelastic collagen fibrils, and osmotic swelling. More details of the model and model validation have been presented in earlier studies[8,9].

Simulations were performed for healthy cartilage and cartilage with early and advanced OA. The geometries of the models were based on average measures of the experimental samples tested in unconfined compression geometry (Table I). Each FE mesh consisted of 288 4-node axisymmetric poroelastic continuum elements, in which the composition based fibril-reinforced poroviscoelastic material with swelling capabilities was programmed using the user-defined material definition (UMAT) in Abaqus 6.5 (Dassault Systémes, Providence, RI).

The depth-dependent composition and structure for the samples were taken from the microscopic and biochemical analyses, similarly as described in earlier studies[8,20–22]. Depth-dependent collagen and PG solid mass fraction, water fraction, collagen orientation and fixed charge density were implemented in the models as an average values obtained from the experiments (see details from our earlier study[8]). In order to clarify the effect of the depth-wise compositional and structural changes in OA on the mechanical behaviour of the samples, a single step dynamic compression of 2 N in unconfined compression was simulated, and the depth-wise axial local tissue strains were analyzed.

STATISTICAL TESTS

Differences in the depth-wise Amide I and Carbohydrate absorptions, and collagen fibril orientation were statistically examined. The depth-wise profiles of all samples were interpolated to 100 points and, subsequently, statistical point-by-point comparison between different OA groups (normal vs early OA, normal vs advanced OA and early OA vs advanced OA) were carried out.

The mixed linear model was chosen for statistical comparisons between the groups[23]. The benefit of this model is that samples with potential interrelations, i.e., dependence between the samples prepared from the same location within patella or between the samples from the same cadaver, can be reliably compared. In the model, OA group and sample location within the patella were set as fixed variables, and the cadaver was coded as the random variable. Restricted maximum Likelihood (REML) estimation was used in the model. Furthermore, estimated means for the different groups (normal, early OA, advanced OA) were obtained from the fitted model, and the main effects between the groups were compared. 95% confidence intervals for differences with Least Significant Difference (LSD) adjustment were finally presented. Statistical analyses were carried out using SPSS (ver. 16, SPSS Inc., Chicago, IL), which was operated with a Matlab script (ver. 7.2, MathWorks Inc., Natick, MA).

Results

Qualitatively, Amide I and Carbohydrate absorptions decreased progressively during OA progression (Fig. 1). In early OA, especially in the superficial and middle zones, changes in Amide I and Carbohydrate absorptions were observed. In advanced OA, significant reduction in Amide I and Carbohydrate absorptions could be seen in all layers.

The collagen fibrils in the superficial zone of cartilage were more disorganized, i.e., less parallel to the surface, in OA samples than in healthy samples ($P < 0.05$), while the changes in the collagen orientation in the deeper zones

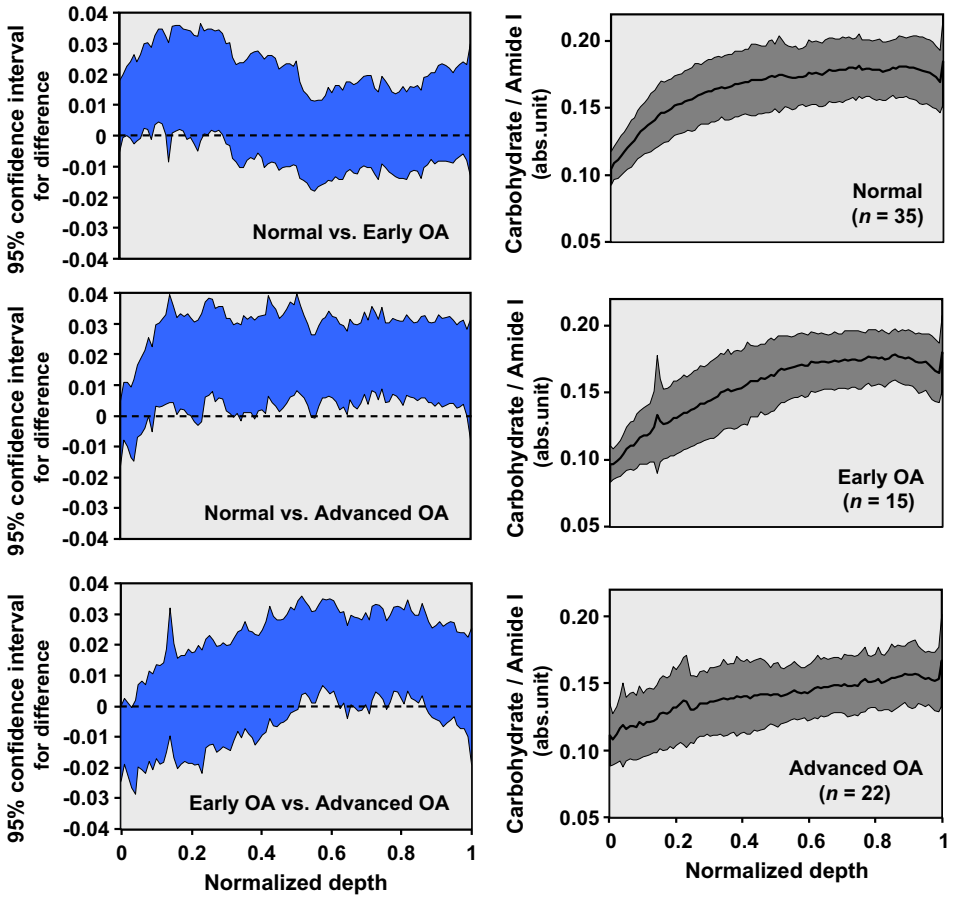

Fig. 4. Right column: Mean values (±SD) of the normalized Carbohydrate region absorption, i.e., another estimate of the PG content, in different groups (normal, early OA and advanced OA) as a function of relative tissue depth. FTIRI was used for the measurements and relative absorption was quantified by normalizing the area under the spectrum in Carbohydrate region (1140–985 cm^{-1}) with the area under the spectrum in Amide I peak (1585–1720 cm^{-1}). Left column: 95% confidence intervals for the difference between estimated means of the normalized Carbohydrate region absorption in different OA groups (normal vs early OA, normal vs advanced OA and early OA vs advanced OA). Mixed linear model was used in the statistical comparisons. In graphs, confidence intervals are shown as a function of relative depth of the tissue.

of cartilage were minor ($P > 0.05$) as OA progressed (Fig. 2). However, in the very deep zone of cartilage, a significant ($P < 0.05$) change in the collagen orientation between the normal and advanced OA groups was observed (Fig. 2).

Similarly to the collagen orientation, Carbohydrate region absorption was reduced in the superficial and middle zones in early OA ($P < 0.05$), as quantified with both directly from the Carbohydrate region and from the ratio of Carbohydrate to Amide I (Figs. 3 and 4) . As OA progressed to the advanced stage, major reduction ($P < 0.05$) in Carbohydrate region absorption in the deeper tissue was observed (Figs. 3 and 4). The samples with advanced OA showed a significant ($P < 0.05$) reduction in the Carbohydrate region absorption only in the middle/deep zone, as compared to the samples with early OA (Figs. 3 and 4). Furthermore, statistical analysis indicated that the ratio of Carbohydrate to Amide I exhibits larger confidence intervals than the direct area of the Carbohydrate region (Figs. 3 and 4).

No statistically significant ($P > 0.05$) reduction in the Amide I absorption was observed in early OA (Fig. 5), as compared to normal cartilage. However, as OA progressed to the advanced stage, a significant ($P < 0.05$) reduction in the Amide I absorption, compared to normal cartilage samples, was observed throughout the tissue depth (Fig. 5). The samples with advanced OA showed a significant ($P < 0.05$) reduction in the Amide I absorption only in the middle zone, as compared to the samples with early OA (Figs. 3 and 4).

After implementing the depth-dependent structural and compositional information of the samples into the FE models (Table I, Figs. 2, 3, 5), the simulated spatial strains of the tissue under a constant impact stress increased progressively as OA progressed (Figs. 2, 3, 5, 6). The increase in local tissue strains during OA progression was similar through the tissue depth.

Discussion

FTIRI and ePLM were used to characterize changes in composition and structure of human articular cartilage during OA progression. Further, structure and composition of cartilage samples with normal, early OA and advanced OA were implemented into the composition-based FE models, and local tissue strains were predicted under impact type of loading. Collagen fibril orientation (as estimated with ePLM) and PG content (as estimated with FTIRI) in the

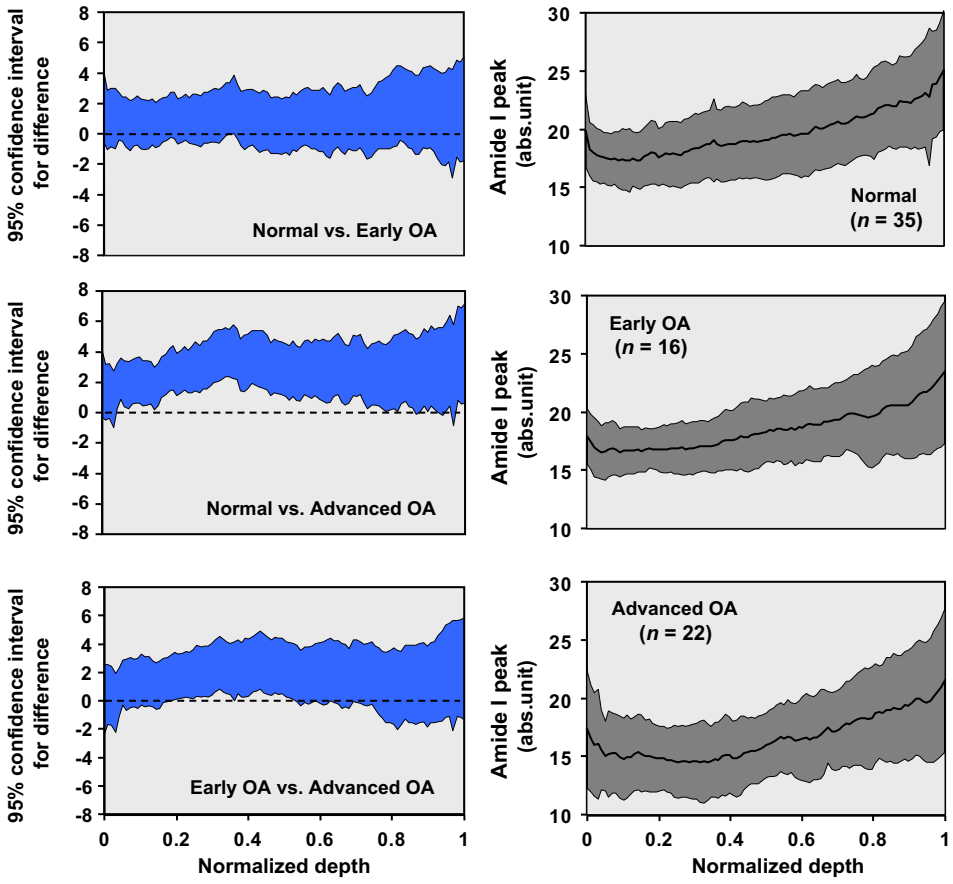

Fig. 5. Right column: Mean values (±SD) of the Amide I absorption, i.e., estimate of the collagen content, in different groups (normal, early OA and advanced OA) as a function of relative tissue depth. FTIRI was used for the measurements and absorption was quantified directly from the area under the spectrum in Amide I peak (1585−1720 cm^{-1}). Left column: 95% confidence intervals for the difference between estimated means of the Amide I absorption in different OA groups (normal vs early OA, normal vs advanced OA and early OA vs advanced OA). Mixed linear model was used in the statistical comparisons. In graphs, confidence intervals are shown as a function of relative depth of the tissue.

superficial tissue changed significantly in early OA compared to normal cartilage. Instead, collagen content (as estimated with FTIRI) showed no decrease until at the advanced stage of OA. Local tissue strains of the numerical models increased substantially as OA progressed.

It is generally accepted that first signs of OA are the fibrillation of articular cartilage surface[3,4,6,7] and PG depletion from the superficial zone of cartilage[1–5]. Our findings clearly support the former sign, as the collagen fibrils were significantly more disorganized (oriented less parallel to the surface) just beneath the surface in early OA than in normal cartilage. The latter sign was also consistent with the earlier studies, but in addition, significant changes in the PG content were found in the middle zone of cartilage. Interestingly, in cartilage with the advanced OA, the superficial PG content was not substantially reduced, but rather the deeper zones experienced major PG depletion. This finding may be due to the fact that the superficial zone could be partly or completely vanished (due to mechanical wear of the tissue) in severely osteoarthritic tissue. This was also supported by our histological evaluation of OARSI grade, in which cartilage matrix loss in the superficial layer was observed especially in samples with high OA grades. Thus, this kind of analysis might not compare exactly the same depth-wise location between the normal tissue and advanced OA. Therefore, one should be careful when drawing conclusions about the superficial zone comparisons between the normal and advanced OA groups.

In the present study, two different approaches were used for the estimation of the PG content, i.e., directly from the integrated area of the Carbohydrate region and using the ratio of Carbohydrate region and Amide I peak. Both of these methods have been shown to correlate with the histologically or biochemically determined PG content[14,15]. The benefit of using the ratio parameter is that the result should not be dependent on the variation in thickness between the microscopic sections[15]. When comparing these two methods for the estimation of PG content, larger reduction of absorption along OA progression was revealed from the integrated area of the Carbohydrate region alone, as compared to the ratio of Carbohydrate and Amide I. Furthermore, the shape of the mean depth-wise absorption profiles was slightly different between the methods. The statistical analysis indicated that the ratio parameter exhibits larger confidence intervals than the Carbohydrate region alone. It is also notable that the results with the ratio parameter are always relative, and the changes in Amide I peak might, in principle, induce uncertainty in the depth-wise results. Although main statistical conclusions remained the same with both methods, it seems that the ratio parameter possess more uncertainty than the direct area of the Carbohydrate region. Consequently, the use of the ratio

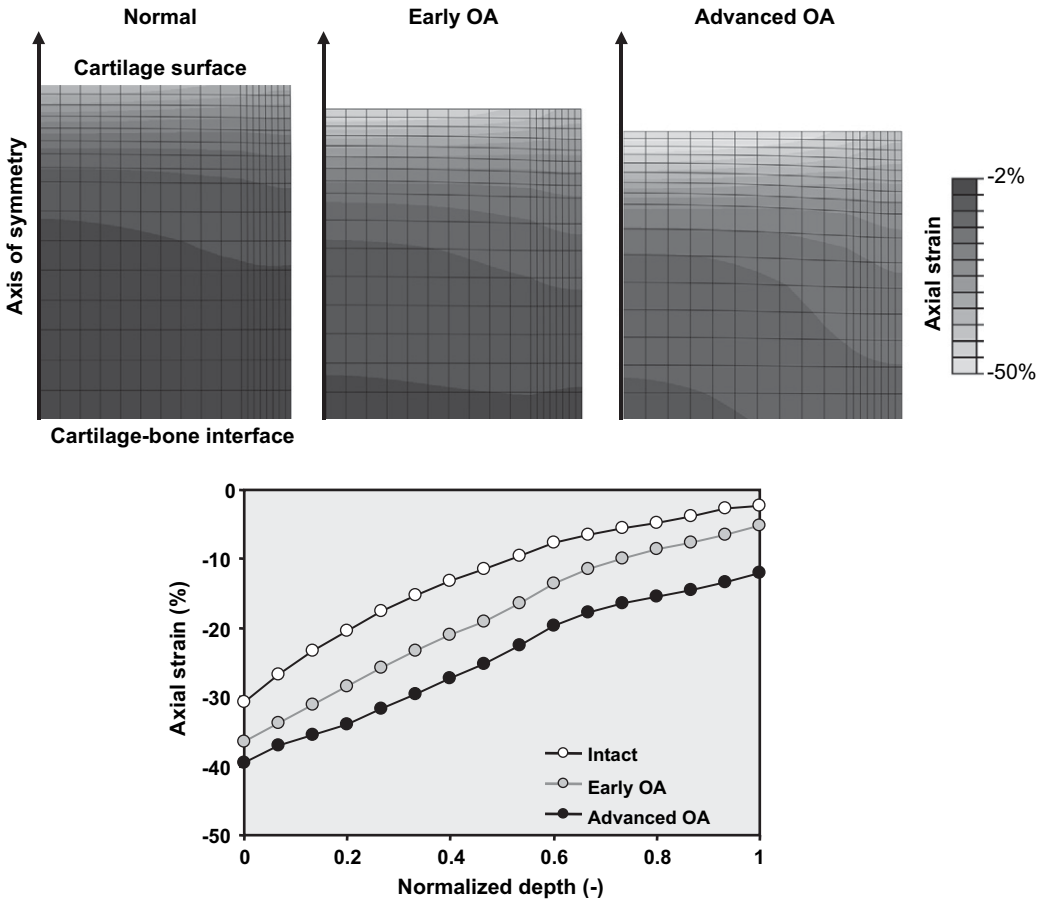

Fig. 6. Spatial deformation (upper part) and local tissue strain (lower part) of normal, early OA and advanced OA samples, as predicted with the composition based fibril-reinforced poroviscoelastic model mesh of articular cartilage. In the simulations of the axisymmetric models, a constant contact stress of 2 N was applied for the samples in unconfined compression geometry and, subsequently, local tissue strains were analyzed. More details of the composition-based model and its input parameters can be seen from the text, Figs. 2, 3 and 5, Table I, and Julkunen *et al.*[8] 0 = Cartilage surface and 1 = Cartilage-bone interface.

parameter is not optimal for the estimation of PG content in the cartilage tissue. Nevertheless, these two current methods, and probably more developed and specific FTIRI analysis methods[24], should be further validated in the future studies.

Collagen content of articular cartilage has been traditionally determined using biochemical analysis. It has been reported that the collagen content would decrease in early OA[25–28]. It has also been suggested that the collagen content would remain unchanged in early OA[29]. Further, Guilak *et al.* (1994) reported that the biochemically determined collagen content in a canine model of early OA is decreased when normalized to the wet weight of the tissue, but remain the same when normalized to the dry weight[1]. Thus, there is an obvious contradiction between the biochemical results, and it is difficult to draw accurate conclusions on changes in the collagen content in OA. With regard to the microscopic FTIR estimation of the collagen content, Bi *et al.* (2006) reported that the collagen content in human articular cartilage decreases in early OA especially at the superficial layer.[4] However, contradictory results have also been published using FTIRI[5]. According to the results of the present study, including comprehensive depth-wise point-by-point statistical comparisons, Amide I absorption in any layer was not found to reduce in early OA, but only in advanced stage of OA compared to normal. The explanation for this may be the slower degradation process of the collagen network during the development of OA, i.e., occurring significantly only in the advanced stage. It is also notable that, similarly with the superficial PG content, the superficial collagen content in advanced OA was not substantially reduced. As discussed earlier, partly or completely missing superficial zone could explain this.

Even though change in the superficial collagen orientation, which may reflect collagen disorganization[30], occurred in the early stage of OA, as characterized by ePLM, the present results suggest that the collagen fibrils were not shattered and released into the joint space, or enzymatic degradation had not significantly reduced the collagen dimensions and content. Consequently, our results suggest that the mechanism behind the collagen degradation and disorganization in early OA may be purely mechanical. During joint loading, possibly, the reduced lubrication[31,32] between cartilage surfaces may at some point change the collagen orientation and organization. Yet, the fibrils may still be attached to the tissue. These speculations about the mechanical cause of the disorganization of collagen network in early OA are also supported by the reports on cartilage surface roughness, which has been shown to increase in early OA[33]. It is also possible that wear of the

articular cartilage surface contributed to the reduced tissue thickness, while the rest of the tissue may still have been fairly intact. When OA progresses to more advanced stages, mechanical loading with further reduced lubrication, increased wear and enzymatic degradative mechanisms may then break up fibrils into pieces and reduce fibril dimensions. As the collagen fibrils get narrower and loose fibril pieces escape from the tissue, the collagen content inevitably reduces, as was observed for the samples with advanced OA. This issue has not been brought up into attention and needs still more research to be thoroughly clarified.

One of the limitations of the present study was the potential site-dependent variation and interrelations of the samples. The best way to avoid this limitation would have been to study different sites independently. However, as we had only a limited number of human cadavers ($n = 14$), the results would not have had enough statistical power. Consequently, mixed linear model was used, which enables the comparison between groups taking into account the interrelations between individual samples. In this study, these interrelations include the dependency of the sample location and of the human cadaver. As a limitation of this approach, we cannot make any site-dependent conclusions from the results. However, this approach allows us to make general conclusions of the structural and compositional changes during OA progression.

The present FE model, able to describe realistically cartilage biomechanics solely based on tissue structure and composition, clearly indicated an increase in local tissue strains as OA progressed. Experimentally, decrease in the collagen content was pretty uniform through the tissue depth. This is probably the reason for the concurrent uniform change in strain. Furthermore, the PG content decreased significantly in the superficial zone in early OA but not between early and advanced OA. That may explain the lower change in strain between early and advanced OA samples in the superficial tissue, as compared to that between normal and early OA samples. Conversely, the change in strain was larger in the deep zone from early OA to advanced OA, as compared to the change from normal to early OA samples. This may be due to the statistically significant change in the PG content in the deep zone in the advanced stage of OA.

The present modelling results suggests that similar loading of a joint with OA cartilage than that of a joint with healthy cartilage could create an increased risk of further tissue failure and cell death[34–36]. The presented model presents an early step toward estimation of tissue mechanics from the noninvasive imaging techniques, such as quantitative (MRI). Combination of MRI based joint geometry and tissue composition and structure with the composition-based FE model could provide a novel tool for the diagnostics of articular cartilage quality and OA. Further, possible failure points in cartilage, related to excessive stresses and strains, e.g., in athletes with joint injuries could be addressed. This kind of diagnostic tool could provide more effective strategies for OA prevention or slowing down the OA progression. Naturally, this means that clinical MRI devices and measurement techniques need to be further developed and validated before this is realistic, and more studies comparing microscopic analyses (e.g., FTIRI), MRI and mechanical properties of articular cartilage should be conducted in the future.

To conclude, this is the first study with depth-wise statistical comparisons of structure and composition of human articular cartilage during OA, including also relatively large sample number in groups ($n \geq 15$). The results of this study suggest that early changes in cartilage during OA include change in the orientation of the superficial collagen fibrils and reduction of PG content in the superficial and middle zones. Possibly, disorganized collagen fibrils in the superficial zone open the pathway for the PG molecules in the superficial and middle zones to escape from the tissue through the surface. This escape of cartilage macromolecules may be facilitated by the altered tissue resistance to loading, as shown by the FE-model. In contrast to PG loss, collagen content did not change significantly in any zone in early OA. This supports the idea that mechanical causes are mainly responsible for the fibrillation of the cartilage surface at the early stage of OA. However, these results are first of its kind, and more investigations are needed to support the presented hypothesis.

Conflict of interest

Authors have no conflicts of interest.

Acknowledgments

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