INTRODUCTION: Structure and composition of articular cartilage as well as metabolic activity of chondrocytes (cartilage cells) change in osteoarthritis (OA) [1]. Among these changes, cell volume has been reported to increase [1,2]. Furthermore, the structure, composition and mechanical properties of the pericellular matrix (PCM) alter in OA [3]. However, the role of PCM in modulating volumetric and morphological changes of chondrocytes in OA is poorly known.

Collagen and proteoglycan (PG) contents of the PCM in the deep zone of human OA articular cartilage have been clarified using Fourier Transform Infrared Imaging (FTIRI) [4]. However, changes in the composition of the PCM in the superficial zone of human cartilage during OA progression are currently unknown.

In this study, morphology of the superficial zone chondrocytes was investigated in intact and degenerated human articular cartilage using light microscope. Further, FTIRI and digital densitometry (DD) were utilized to determine the PCM composition. We hypothesize that the collagen of the PCM is related to cell morphology in OA.

METHODS: Human patellae (n=14) were obtained from right knees of cadaveric donors (12 males, 2 females, age 55±18 years) [5]. Intact and spontaneously degenerated samples were collected from six locations in each patella (superomedial, superolateral, central medial, central lateral, inferomedial and inferolateral; n=79). The samples were graded according to OARSI criteria [5,6] and were divided into three groups: Normal (n=34, OARSI=0), Early OA (n=17, OARSI=1-1.5) and Advanced OA (n=22, OARSI=2-4.5).

For the analysis of cell dimensions and estimation of PG content in the PCM, 3 µm thick histological Safranin-O stained sections were prepared. From each microscopic section, a 40x images of four to five cells in the superficial zone (<10% of the sample thickness) were captured with the light microscope (Axio Imager M2, Zeiss, Germany). Height, width and aspect ratio (height/width) of the cells were analyzed from the images (Fig. 1). Further, area of the cells was calculated by assuming the elliptical cell shape.

For the estimation of the PG content of the PCM, 25x images of four to five cells in the superficial zone were captured using DD (Photometrics CH250 ltd.,Tucson, Arizona, USA). A rectangular profile (height 6 µm and width 69 µm) was manually drawn perpendicular and parallel to the cartilage surface by including one superficial zone cell in the middle of each profile (5 cells from each sample). Profiles were averaged for each cell, normalized by the values at the edge of each profile and finally the maximum optical density values at both sides around each chondrocyte in the PCM were manually selected to represent the PG content of the PCM.

For the estimation of the collagen content, all samples were measured with FTIRI (PerkinElmer Spectrum Spotlight 300, Perkin Elmer inc., Shelton, CT, USA) using the pixel size of 6.25 µm. The Amide I absorption peak (1720-1585 cm⁻¹) in the vicinity of the chondrocytes was used to estimate the collagen content in the PCM [7]. In the FTIRI analyses, a single line profiles were manually drawn through the individual superficial zone cells (5 cells from each sample) perpendicular and parallel to the sample surface. The line profiles were normalized by the parameter values at the edge of each profile.

Statistical differences in height, width, aspect ratio and area of the chondrocytes, as well as normalized PG and collagen contents in the PCM between OA groups were analyzed with the linear mixed model using the SPSS software (SPSS 14.0, Chicago, IL, USA).

RESULTS: Significant changes in chondrocyte morphology were observed during OA progression (Fig. 1, Table 1). Specifically, the cell aspect ratio was significantly greater (p<0.01) in advanced OA group compared to normal and early OA groups (Table 1). Further, the area of chondrocytes enlarged significantly (p<0.05) from normal to early OA and advanced OA samples. The absolute PG content decreased as OA progressed. Mean normalized PG content in the PCM for all groups was 1.29±0.83, however, there was no significant differences between the groups (p>0.05). Normalized amide 1 absorption profiles changed significantly (p<0.05) in the PCM of advanced OA samples (Fig. 2).

DISCUSSION: Significant changes in the morphology of chondrocytes were observed during OA progression. Chondrocytes became larger and changed more spherical especially in the tissue with advanced OA. Simultaneously, the relative collagen distribution of the PCM changed significantly in advanced OA. This is consistent with our recent finding, which suggested that the collagen content of the extracellular matrix (ECM) changes not until at the advanced stage of OA [8], and supports our hypothesis that the collagen of the PCM is related to cell morphology. In contrast, relative PG content of the PCM did not change as OA progressed, even though the cell area increased in early OA and absolute PG content has been shown to dramatically decrease in the ECM in OA in the superficial zone of human cartilage [8]. Although the PG synthesis may be inhibited or accelerated in different stages of OA, the PGs seem to be distributed evenly in the tissue and do not remain more locally around the cells in the PCM.

This study suggests that the collagen content of the PCM is related to cell shape changes in human OA cartilage, while PGs may primarily contribute to the changes in the cell area (and presumably cell volume). By combining the present experimental microscopic methods with the theoretical biomechanical models of cartilage, it is possible to specifically address the roles of collagen and PGs in the PCM on cell morphology in OA. This could then strengthen the present findings.


Table 1. Mean width, aspect ratio and area (±S.D.) of chondrocytes in normal, early OA and advanced OA sample groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Height (µm)</th>
<th>Width (µm)</th>
<th>Aspect ratio</th>
<th>Area (µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7.18±1.09</td>
<td>10.63±1.09</td>
<td>0.69±0.11</td>
<td>60.3±12.61</td>
</tr>
<tr>
<td>Early OA</td>
<td>8.0±1.01*</td>
<td>11.75±1.01*</td>
<td>0.69±0.09</td>
<td>75.05±14.50*</td>
</tr>
<tr>
<td>Advanced OA</td>
<td>8.54±1.16*</td>
<td>10.38±1.16</td>
<td>0.83±0.13*</td>
<td>70.83±19.63*</td>
</tr>
</tbody>
</table>

*p<0.05, significant difference compared to Normal group.

Figure 1. Typical superficial zone chondrocyte in A) normal, B) early OA and C) advanced OA samples. Height and width of the chondrocytes are marked in the figure, and the PCM areas are identified by arrows.

Figure 2. Normalized mean Amide I absorption profiles around the chondrocytes in the superficial zone. *p<0.05, significant difference between Advanced OA and Normal OA or Early OA.