INTRODUCTION: Changes in cellular density with aging in articular cartilage have been reported in several studies (1,2,3). Age-related studies of cell density in humans have not taken into account the lifestyle of these individuals or their genetic backgrounds (3). Early stages of osteoarthritis (OA) development, as well as differences in weight-bearing areas, could also clarify the controversial data of these changes of cellular density in articular cartilage as a function of age (4). In this study we are presenting, under control conditions in an animal model (i.e., rabbits), the changes of cell density in articular cartilage as a function of age, weight-bearing area (i.e. lateral and medial femoral condyle, lateral and medial tibial plateau), and the different articular cartilage layers (i.e. superficial, middle and deep zones). Biochemically total hydration and total glycosaminoglycan (GAG) content of these tissues has been assessed as a function of age.

MATERIALS AND METHODS: Cell viability and cell density: Under institutional animal care and use committee approval, 8 knees from mature (8-18-mo old) New Zealand white rabbits with closed epiphyses and 12 knees from aged (4-5-yr old) rabbits were obtained and dissected. Among them only the joints receiving a macroscopic Grade I of the Outerbridge classification, indicating intact surfaces of the femoral and tibial plateau of articular cartilage, were used for further examination. Cartilage pieces were cut out as full-thickness flaps with a scalpel and minced into 1mm-width coronal sectioned fragments. Cell density and cell viability were evaluated using a fluorescent in situ double staining protocol, followed by confocal microscopic analysis (5). The total number of live (green stained) and dead (red stained) cells in each layer were counted utilizing the image analysis program (NIH image 1.6) and the percent viability then calculated (6). Demarcation lines of each layer were determined by cell size and cell arrangement. Statistical analysis was done with Mann-Whitney’s U test. (p<0.05 or 0.01 was considered a statistically significant difference)

Biochemistry: Total hydration and glycosaminoglycans (GAG) was also assessed (7) on articular cartilage Grade I obtained from the femoral condyle and tibial plateau from 69 knees of mature and aged New Zealand white rabbits. Statistical analysis was done utilizing unpaired t-test with a level of significance of p<0.05.

RESULTS: Joint grading for cell viability and cell density: In aged rabbits 12 out of 12 cartilage from the lateral femoral condyle, 11 out of 12 lateral tibial plateau, 9 out of 12 medial femoral condyle, and 9 out of 12 tibial plateau were Grade I, and were used for subsequent confocal microscopic study. The other 6 cartilage pieces were Grade II, and only 1 out of 12 medial tibial plateau was Grade III. In mature rabbits all the cartilages were Grade I except for two medial tibial plateaus that were evaluated as Grade II.

Biochemistry: The percent hydration and amount of GAGs in the femoral condyle and tibial plateau of articular cartilage is described in Table 1. No statistical differences were observed for femoral condyles in both hydration and GAGs. For the tibial plateau, articular cartilage hydration was the only parameter decreased with statistical significance (p<0.006).

Cell viability: Cell viability in the aged cartilage was lower than mature cartilage, however no statistically significant differences were seen in all the areas. The cell viability of articular cartilage was 96.5% in mature and 94.7% in aged rabbits.

Cell density: In all 3 layers of the 4 regions studied, the cell densities of articular cartilage were significantly lower in the aged cartilage compared to mature cartilage (Table 2). The decrease in cell density was more predominant in the tibia than the femur, with p-values <0.01 in all 3 layers. In the medial tibial plateau, cell densities decreased 68% in the superficial zone, 64% in the middle zone, and 50% in the deep zone (Table 2 & Fig 1).

DISCUSSION: This study demonstrates the cellular distribution in function of area, and zone in articular cartilage of the rabbit knee. This report is to our knowledge the first one, which compares mature versus aged femoral versus tibial, and the three zones of the articular surface in well-controlled animal model. The decrease in cell density which was the greatest in the superficial zone observed in our model, with a high percentage of cell viability, and the no statistical differences in the matrix composition, lead us to speculate that the decrease in cell density with time will affect the degeneration of the matrix, which may be a prelude to the development of osteoarthrosis (OA).

Table 1: Biochemical analysis of rabbit articular cartilage (hydration was expressed as percent and GAGs were expressed as g of hexosamine per mg of dry cartilage tissue).

<table>
<thead>
<tr>
<th></th>
<th>Superficial</th>
<th>Middle</th>
<th>Deep</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mature</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femur</td>
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<td>90.3±2.3</td>
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<td>Tibia</td>
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<td><strong>Aged</strong></td>
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</tr>
<tr>
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<td>44.1±2.6</td>
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<tr>
<td>Tibia</td>
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<td>39.1±2.4</td>
<td>29.1±2.5</td>
</tr>
</tbody>
</table>

Table 2: Cell density of rabbit articular cartilage (10^3/mm^2, ±S.D., *p<0.05, **p<0.01).

REFERENCES:

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