THE CELL DENSITY OF THE SUPERFICIAL LAYER OF ADULT HUMAN ARTICULAR CARTILAGE IS JOINT-SPECIFIC AND IS ALTERED BY AGE AND DEGENERATIVE CHANGES

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Introduction
Degenerative changes of articular cartilage are characterized by destruction and are associated with reduced cellularity. The purpose of the present study is to identify the specific superficial and deep layers chondrocyte density per articular cartilage surface. Furthermore, the hypothesis is tested that degenerative changes upregulate the initial superficial cell density in Collins Grade II.

Materials and Methods
Human adult articular cartilage from 65 synovial joints, including 6 distal humeri, 4 radial heads, 4 ulnar trochleae, 5 distal femora, 4 proximal & 5 distal tibiae, 16 talus domes and 11 talus navicular and 10 talus subtalar facets, was obtained in collaboration with the Regional Organ Bank of Illinois from 13 donors. The age range was 32-75 years. Both normal cartilage and samples with degenerative changes were accepted.

The joints were opened within 24 hours of death and graded on the scale of Collins (1949) as modified by Muehlemann (1997). Horizontal slices of approximately 100µm thickness of the superficial layer were manually dissected from the articular surface. Then, all remaining cartilage was dissected. Tissue was incubated per layer and per joint with pronase (24mg/g cartilage) for 90 minutes and with collagenase p (3 mg/g cartilage) for 18 hours at 37°C.

Following digestion, the chondrocytes were viewed with Hoffman modulation contrast microscopy using a Nikon Diaphot inverted phase microscope. Cell counts were performed three times using a Bright-Line Hemacytometer 0.1mm Deep (Reichert, Buffalo, N.Y.)

Total cell numbers and cell numbers per gram for superficial and deep layers of cartilage were determined for each sample. For statistical analysis Microsoft Excel® data analysis was used to group the data jointly and to calculate arithmetic mean and confidence; the separate-variance t test (welch’s t-test) was used jointly to evaluate the level of significance (p-value, one tail) for superficial vs. deep layers cell density, for damaged cartilage Collins Grades 0-I vs. II and for the age groups 32-34 years vs. 57-75 years.

Results
For all joints, for all age groups and for Collins 0-I and II, the average superficial cell density is higher than the average deep layer density, except for the talus subtalar facet. As the only true concave articular surface of all included joints, the subtalar facet (n=8, Collins 0-I) showed a lower average superficial than average deep chondrocyte density (S: 1.3 x10^6 per gram; D: 3 x10^6 per gram).

In the lower extremity, the highest average superficial chondrocyte density of all joints Collins 0-I show the distal femur (9.4 x10^6 per gram), the distal tibia (8.6 x10^6 per gram) and the proximal tibia (7.8 x10^6 per gram).

The ankle joint Collins 0-I has, all ages included, an average density of 7.5 (x10^6 per gram). While the talus dome of 32-34 ages shows a relatively high superficial density of 8.9 (x10^6 per gram), the superficial density of 57-75 years age is decreased to 6.2 (x10^6 per gram).

The highest average deep layers cell density of the lower extremity of all joints Collins 0-I have the distal femur (4.8 x10^6 per gram), the distal tibia (3.3 x10^6 per gram) and the proximal tibia (3.2 x10^6 per gram). A significant difference of superficial to deep layers average cell density of the lower and upper extremity could only be found in the distal femur (in Collins 0-I is S:D=9:4; 4.8 x10^6 per gram, p=0.0484*); in Collins II is S:D=15:7; 3.1 (x10^6 per gram, p=0.0219*). In and in the talus dome (in Collins 0-I and age 32-75 is S:D=7:5; 6.2 (x10^6 per gram, p=0.0449*); in Collins I and age 32-34 is S:D=8:9; 4.7 (x10^6 per gram, p=0.0449*); in Collins 0-I and age 57-75 is S:D=6:2; 3.6 (x10^6 per gram, p=0.0348*)).

In the elbow joint, the highest average superficial cell density shows the distal humerus Collins 0 (9.17 x10^6 per gram), while the radial head and the trochlea have only a superficial cell density of 4.05 and 4.75 (x10^6 per gram). A significant difference of superficial to deep layers average cell density has not been found in the elbow joint.

Discussion
Each joint surface has a unique, joint-specific superficial and deep cell density and a fixed ratio of those. In the lower extremity, the cell densities of both, superficial and deep layers, increase in the same order from talus dome, proximal tibia, distal tibia to distal femur, regardless of different age groups or Collins Grades of the talus dome.

The distal femur Collins 0-I superficial layer density is twice as high as the deep layer density; with progression of degenerative changes, this density ratio increases to a more than five times higher superficial than deep density in Collins II. Comparing only superficial layers, the increase in density from Collins 0-I to Collins II is 150%.

The deep layer cell density of the distal femur is not significantly affected by degenerative changes.

Considering the talus dome, the ratio of superficial to deep layer cell density is only slightly changed by age or progression of degenerative changes from Collins 0-I to II. However, the superficial cell density is decreased with age from 8.9 to 6.2 (x10^6 per gram), and is increased from 6.2 to 11.6 (x10^6 per gram) in Collins II; this was only performed in the group of older aged donors since younger aged specimen of Collins II were not available.

In the ankle joint, the deep layer cell density is not affected significantly by age or progression of degenerative changes.

Physiologically, the cell density of superficial and deep layers of adult human articular cartilage is joint-specific; the superficial layer cell density decreases with age and increases with progressive degenerative changes (Collins 0-I to II). The deep layer cell density is not significantly affected by age or degeneration (Collins 0-I to II).

References
1: Aydelotte & Kuettner, 1988, Connect Tissue Res, 18, 205-222