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## Proliferative re-modeling of the spatial organization of human superficial chondrocytes distant to focal early osteoarthritis (OA)

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### Abstract

**Objective**—Human superficial chondrocytes show distinct spatial organizations whereas they commonly aggregate near osteoarthritic (OA) fissures. It is not known whether remodeling or destruction of the spatial chondrocyte organization may occur distant to focal (early) OA lesions.

**Methods**—The intact cartilages (condyles, patellofemoral groove, proximal tibia) distant to focal OA lesions of human grade 2 joints were compared to location-matched non-degenerative (grade 0–1) cartilages. Chondrocyte nuclei were stained with propidium iodide and recorded by fluorescence-microscopy in a top-down view. Chondrocyte arrangements were tested for randomness or significant grouping via point pattern analyses (Clark and Evans Aggregation Index), and were correlated with OA grade and surface cell densities.

**Results**—In grade 2 cartilages, superficial chondrocytes were situated in horizontal patterns such as strings, cluster, pairs and singles comparable to non-degenerative cartilage. In the intact cartilages of grade 2 joints, the spatial organization included a novel pattern, consisting of chondrocytes that were aligned in two parallel lines building double strings. These double strings correlated with an increased number of chondrocytes per group ( $p < 0.05$ ), increased corresponding superficial zone cell density ( $p < 0.001$ ), and were observed in all grade 2 condyles ( $p < 0.001$ ), some grade 2 tibiae ( $p < 0.05$ ) but never in grade 0–1 cartilage ( $p < 0.001$ ).

**Conclusion**—The present study is the first to identify a distinct spatial re-organization of human superficial chondrocytes in response to distant early OA lesions and suggests that proliferation had occurred distant to focal early OA. This spatial re-organization may serve to recruit metabolically active units as attempt to repair focal damage.

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Human superficial chondrocytes are grouped into distinct spatial organizational patterns such as singles, pairs, clusters, and strings (1). Those organizational patterns are oriented parallel to the articular surface. In large human joints such as shoulder, elbow, knee and ankle joints, the articular surfaces are typically dominated by only one of these four patterns. In addition, we showed recently that the type of organizational pattern depended on the diarthrodial joint type suggesting that the spatial chondrocyte organization of the superficial zone correlates with a particular joint function. Thus, in intact cartilage, human superficial chondrocytes can build distinct spatial organizational patterns with functional yet unknown relevance (1).

Osteoarthritis (OA) is a degenerative disease of the entire synovial joint and is characterized by two phases: a biosynthetic phase, during which the chondrocytes attempt a repair of the damaged extracellular matrix, followed by a later degradative phase, in which matrix synthesis inhibition and digestion by catabolic enzymes lead to collagen network damage and subsequent matrix fissuring, erosion and loss (2–7). In early OA, the degenerative process appears to begin at the articular surface (8,9) where proliferative chondrocyte clones characteristically form at the margins of zones of fibrillation (10,11). In moderately advanced OA, chondrocyte clones are also found adjacent to surface clefts (12) whereas late stage OA is characterized by chondrocyte aggregation in the middle and deeper cartilage zones (13) in areas where the superficial zone has been lost.

Other than chondrocyte aggregation at the margins of zones of fibrillation and adjacent to surface clefts, it is not known how OA may affect the spatial organization of superficial chondrocytes. In the present study, we hypothesize that specific changes in the spatial chondrocyte organization may also occur distant to focal lesions in early OA. Specifically, we addressed the question whether human superficial chondrocytes experience destruction versus specific remodeling of their horizontally orientated spatial organization in early OA. For three reasons, we concentrate on the remaining intact surface of early OA cartilage with focal damage. First, it is well established that superficial chondrocytes adjacent to fibrillation or fissuring undergo OA-typical aggregation (10). Second, advanced OA-specimens, although they are relatively easy to obtain, likely have lost their superficial zone. Third, we recently showed that the intact remaining cartilage of focally degenerated OA-surfaces shows evidence for a generalized response and active matrix remodeling across the entire surface to repair focal damage (14).

## Material and Methods

### Articular Cartilage

Articular cartilages were obtained from human knee joints. Each articular surface was graded according to Collins, modified by Muehleman (15) using following criteria: grade 0 (intact cartilage), grade 1 (minor surface roughening), grade 2 (fibrillations and fissuring), grade 3 (full defects covering <30% of the articular surface), and grade 4 (full defects covering >30% of the articular surface). Only joints were included, for which both articulating surfaces received the same grading (0–1 or 2). For further details see supplement.

**Non-degenerate cartilage**—For analyses of non-degenerate cartilages (grade 0–1), tissues from 8 human donors were obtained from the distal femur (condyles; patellofemoral groove) and proximal tibia.

**Remaining, intact cartilage of grade 2 joints with focal OA lesions**—For studies of the remaining, intact cartilage of joints with focal OA (grade 2), microscopically intact cartilage from 5 donors with focal lesions of the bearing region of the medial condyle was

dissected from the weight bearing region of the lateral condyle, patellofemoral groove, and proximal tibia. Samples were location-matched with non-degenerate samples. Although we do not know whether the donors were diagnosed with clinical OA, their lesions were similar to those of early OA lesions. For simplification, we henceforth use the term early OA-lesion.

**Fissured cartilage in proximity of focal OA lesions**—Selected samples within proximity of focal lesions were analyzed to assess chondrocyte aggregation near surface fissures.

### Fluorescence Microscopy

Visualization of the spatial chondrocyte organization was achieved as previously described (1). Samples of approximately 200–300µm thickness (16) and 1cm<sup>2</sup> area were dissected from each joint surface without further sectioning to preserve intact chondrocyte patterns. Samples were stained as previously described with propidium iodide (1), and viewed perpendicular to the articular surface, providing a top-down view. Two-dimensional images were digitally recorded for each surface (Nikon Eclipse TE200, magnifications 10×, 20×) at the sample center as single topographical location.

### Quantitative Image Analysis

Images were digitally filtered to exclude cells that were in different planes. Based on spatial arrangement, groups were classified as singles, pairs, clusters, strings and double strings. Pairs were defined as two neighboring chondrocytes, clusters as circular arrangements of chondrocytes, strings as chondrocytes aligned in lines, and double strings as chondrocytes aligned in two parallel lines in close proximity (20–30µm). Based on proximity, the depicted nuclei were grouped numerically. The amounts of cells that were situated in numerically defined groups or spatial patterns were determined to calculate the percentages of all cells that were situated within those groups or patterns. We also calculated the total amount of cells per image.

### Point Pattern Analysis

To determine if the spatial arrangements were random, homogeneous or significantly grouped in a point pattern context (17), the nearest neighbor method was used (1,18). Nuclear centroids (geometrical nucleus center) were determined. The mean nearest neighbor distances between the centroids of two neighboring chondrocytes were calculated. The ratio of observed values of the distance to the nearest neighbor and their expected values in an assumed homogeneous distribution was used to calculate the Clark and Evans Aggregation Index (R) to determine the presence or absence of grouping (19).

### Correlation of Chondrocyte Patterns with Degenerative Changes

To assess whether the observed chondrocyte patterns correlated with the OA grades, the percentage of cells situated within each pattern and the OA grades were tested with a Spearman Rank Order Correlation. We also assessed the correlations of the decrease or increase in the cell numbers per chondrocyte group with the OA grades.

### Cell Density

To assess changes in the cell densities, we isolated the chondrocytes using an established method (1,20). Briefly, the entire articular surface was dissected to a depth of 200µm and defined as superficial zone. The remaining non-calcified cartilage was defined as deeper zones. After the chondrocytes were released, the cell densities were calculated as the number of chondrocytes per gram cartilage wet weight (1). It has been suggested that the cellular

yield, especially from human cartilage, is relatively low and may depend on the extent of matrix degeneration and thus be skewed between different groups. To compare our results to previous studies, we calculated the ratio between the densities of the superficial and the deeper zones and found that it was comparable to those of other studies (21–23).

## Histology

Serial sections of non-degenerate joint surfaces and of surfaces with early OA were stained with cresyl violet and viewed with bright-field light microscopy or stained with picrosirius red and viewed with polarized light (24).

To demonstrate the OA-grading, (supplement) Fig. S5AB and S6A-D display non-degenerate cartilage with an intact surface or with minimal surface discontinuities (25). Fig. S5C displays grade 2 focal lesions extending into the deep zones and surrounded by an intact surface (Fig. S5AB; Fig. S6A-D).

## Statistical Analysis

Data are presented as mean±SEM (standard error of the mean) of  $n$  individual experiments and were subjected to one way ANOVA or ANOVA on Ranks, followed by Holm-Sidak or Dunn's post-hoc-tests. Spearman Rank Order Correlation tests were performed to test for correlations between numerical and spatial changes and OA stage. Differences were considered significant at  $p \leq 0.05$ . All tests were performed using SigmaStat 3.1 (Systat Software Inc., CA).

## Results

### Fluorescence Microscopy

To analyze the spatial arrangement of the horizontally aligned superficial chondrocytes, we photographed the chondrocytes from top-down views of the entire depth of the uncut, intact superficial zone. In all photographs, the chondrocytes showed a propidium iodide dye accumulation suggesting identifiable, intact nuclei (Figure 1A-D). In non-degenerative cartilages (grade 0–1), superficial chondrocytes were organized in distinct patterns that were aligned parallel to the articular surface as previously described (1). We observed **strings**, containing three or more cells in a straight line (Figure 1A); **clusters** (Figure 1C), containing three or more cells in a circle or ellipse; **pairs**, containing two cells in proximity, or **single** chondrocytes that were not in proximity to other chondrocytes (Figure 1A, C). There was no difficulty in distinguishing between the patterns. However, in some intact cartilages of grade 2 joints, we identified an additional pattern that was not observed previously: chondrocytes were situated in double strings consisting of two cellular lines in close proximity. This pattern of **double strings** was only observed in the intact articular surfaces of the condyles and the proximal tibia of grade 2 joints, but not in the patellofemoral groove or in non-degenerate joints (Figure 1B, D). When we further investigated the intact cartilages of grade 2 joints, we also found chondrocyte patterns that were typical for cartilage without degeneration (Figure 1B, D); however, the amount of chondrocytes in almost all patterns was decreased in favor of the novel pattern (Figure 2).

Articular ECM without fluorescent signals, suggesting areas of cellular apoptosis or necrosis, was never observed. Cartilage taken near grade 2 focal lesions revealed the presence of large chondrocyte aggregations (Figure 1E, F) that are well-documented (12, 13). We were unable to establish any organizational patterns within these areas of lesion-associated surface aggregations (see *Point Pattern Analysis*); hence we use the term 'unorganized' aggregation. These areas of unorganized aggregation were only observed in the cartilage shoulders of grade 2 focal lesions but never within the remaining, intact

cartilage. Unorganized aggregation was never observed in co-occurrence with the previously reported organizational patterns (1). There was no difficulty in distinguishing between the unorganized OA aggregation and the organized spatial “cluster” pattern because the latter contained three or more cells in a symmetrical circle or ellipse.

### Quantitative Image Analysis

To unravel differences in the amount of chondrocytes that were situated within given patterns, we performed quantitative analyses of the top-down views. In intact condylar cartilage of grade 2 joints,  $67.62 \pm 4.18\%$  percent of all superficial chondrocytes were situated within cellular double strings; the percentage of cells in single strings decreased from  $51.25 \pm 4.94\%$  in non-degenerate cartilage to  $3.73 \pm 1.55\%$  in intact cartilage of grade 2 joints (Figure 2,  $p < 0.001$ ). In non-degenerate cartilage of the condyles, chondrocytes were never situated within double strings, whereas each sample of the intact condylar cartilage of grade 2 joints showed to some extent double strings ( $p < 0.001$ ).

In the proximal tibia, double strings were also observed but not consistently in all samples. The percentage of double strings in intact tibia cartilage of grade 2 joints was  $16.49 \pm 10.29\%$ , whereas double strings were never situated in non-degenerate tibia cartilage. In addition, we observed in the proximal tibia that the percentage of cells in clusters were almost doubled in the intact cartilage of grade 2 joints compared to non-degenerate joints ( $p < 0.01$ ).

The decrease of some ‘classical’ patterns in favor of the novel double strings was significant for single and paired chondrocytes in both the intact cartilage of the condyles and the proximal tibia of grade 2 joints ( $p < 0.05$ ). Only in those locations, in which we observed the presence of double strings, we also observed the decrease of singles and pairs. In the patellofemoral groove, double strings were never present (non-degenerate cartilage, intact cartilage of grade 2 joints); accordingly, no changes of the ‘classical’ patterns were observed. Significant differences between joint surfaces and OA grades are summarized in Table I.

For those intact cartilages of grade 2 joints, which showed changes in their organizational patterns, we also analyzed numerical changes in the amount of chondrocytes per group. In non-degenerate cartilage of the femoral condyles, the largest observed groups contained 6 to 8 cells, in which  $14.16 \pm 6.80\%$  of the superficial chondrocytes were situated ( $p < 0.001$ ). In the intact cartilages of grade 2 joints, the largest cell groups contained 9 to 12 cells, and  $49.06 \pm 3.48\%$  of superficial cells were situated in these larger groups ( $p < 0.001$ ). In the non-degenerate proximal tibia, the largest observed groups contained 6 to 8 cells, in which  $4.99 \pm 1.43\%$  of the superficial chondrocytes were situated ( $p < 0.001$ ). In the intact proximal tibia cartilage of grade 2 joints, the largest cell groups contained 9 to 12 cells, in which  $30.52 \pm 8.56\%$  of superficial cells were situated ( $p < 0.001$ ) (Figure 3).

These numerical changes were associated with changes in the chondrocyte patterns. In condylar cartilages, the cellular groups became larger and predominantly wider but not longer because the pattern of single strings was modified to two parallel strings (double strings). In contrast, clustered groups became larger and consisted of more cells but did not change their pattern.

We also compared the total numbers of cells per image to assess differences in the total image cellularity between non-degenerate cartilage and intact cartilage of grade 2 joints. However, we were unable to establish differences based on the total image cellularity; therefore, we analyzed in addition the cell densities, which were calculated as the number of released chondrocytes per gram wet weight of the dissected tissues.

## Point Pattern Analysis

To assess whether the spatial arrangement of cells was random, homogeneous or grouped, we performed a point pattern analysis by calculating the Clark and Evans Aggregation Index (R) (19) of observed values of the nearest neighbor distances and their expected values in an assumed homogeneous distribution. Briefly, a ratio = 1 describes a random distribution; a ratio <1 describes a grouped distribution, while a ratio >1 defines a homogeneous distribution. For chondrocytes forming double strings, R was 0.5585 ( $p<0.0001$ ) indicating a significant grouping of the superficial chondrocytes in the intact cartilage of grade 2 articular surfaces. For chondrocytes forming strings, clusters, pairs, and singles, we also confirmed a significant grouping as previously described (1). However, we were not able to show a grouped distribution for areas of lesion-associated surface aggregations.

To analyze differences in the chondrocyte proximity, we compared the mean nearest neighbor distances but no significant differences were found between non-degenerate cartilage and intact cartilage of grade 2 joints or between the condyles, the patellofemoral groove and the proximal tibia ( $p>0.05$ ).

## Correlation of Chondrocyte Patterns with early OA

Because we observed double strings in all intact condylar cartilages of grade 2 joints but never in grade 0–1, correlation analyses were performed to show that the presence of double strings was associated with early OA. We were able to correlate early OA with double strings in condylar ( $p<0.001$ ) and tibial ( $p<0.05$ ) cartilage. We also correlated early OA with an increase of chondrocyte numbers in groups of 9–12 cells in condylar and tibial cartilages (larger groups were not observed;  $p<0.05$ ). In the tibia, early OA also correlated with the decrease of single ( $p<0.001$ ) and paired chondrocytes ( $p<0.05$ ).

## Cell Density

To assess early OA-associated cell density changes, we compared the grade 0–1 with the grade 2 cell densities. In the distal femur, the superficial cell density increased by 63 % from grade 0–1 to grade 2 ( $p<0.05$ ). In contrast, the proximal tibia cell densities were not changed (Figure 4).

## Discussion

The present study analyzed the spatial organization of human superficial chondrocytes in the remaining intact cartilage of articular surfaces with focal, early and OA. Comparing the organizational patterns of chondrocytes distant to focal lesions with those observed in normal non-degenerative cartilage, the present study examined whether superficial chondrocytes experience destruction versus specific remodeling of their horizontal organization in early OA.

One of the important observations of the present study was that superficial chondrocytes, when distant to focal lesions in early OA, maintained a distinct spatial organization comparable to non-degenerative cartilage. In the intact cartilages of grade 2 joints, we observed similar horizontal patterns including chondrocyte strings, cluster, pairs and singles as we previously reported for intact cartilages (1). Most importantly, the spatial organization of all intact condylar and some tibia but not patellofemoral groove cartilages of grade 2 joints was greatly modified in two ways. First, in early OA, we identified a novel spatial pattern in the superficial chondrocyte organization. This novel pattern consisted of chondrocytes that were aligned in two parallel lines in close proximity building double string patterns. In addition, the percentages, in which some of the ‘classical’ chondrocyte patterns occurred, had drastically changed in favor of the novel pattern. In each condylar and



some tibia grade 2 samples, the novel spatial pattern co-existed with the organizational patterns of normal, non-degenerative cartilage. To the authors' knowledge, the present study is the first to identify a distinct spatial re-organization of human superficial chondrocytes in response to early OA that results in significantly different organizational patterns compared to normal non-degenerative cartilage.

The present data unquestionably raises the question whether cellular proliferation may be responsible for the observed increase in the superficial chondrocyte numbers and the spatial remodeling of the cellular organization in early OA. Although proliferation of chondrocytes is controversial, many studies have shown that chondrocytes are in fact mitotically active (2,26–33). In OA, the fibrillation- or lesion-associated cellular aggregation is thought to be related to chondrocyte proliferation (2,10–12,34). Proliferative factors such as basic fibroblast growth factor (bFGF) from the synovial fluid (35), cell shape changes, matrix depletion and disruption may induce proliferative cellular aggregation (25,36). A study on advanced OA specimens doubted the existence of chondrocyte cell division and, consequently, the existence of cell proliferation in OA (33). Observing mitoses as direct evidence of proliferation, they reported the absence of mitotic figures in advanced OA cartilage. Nevertheless, they concluded that the induction of cell division in adult and OA cartilage remains an open subject due to the existence of factors essential for cell division (33). Most importantly, they considered chondrocyte proliferation to be likely in early OA (33). Another study on cryoinjured cartilage analyzed the number of centrosomes as evidence for cells undergoing a cell cycle. They reported that 0.7 % of cryo-injured chondrocytes had two centrosomes and concluded that adult chondrocytes retain the ability to repopulate the matrix, possibly as an initial stage of cartilage regeneration (32). In the present study, we showed that both the numbers of chondrocytes per cell group in the grade 2 condyles and the corresponding superficial zone cell density of the grade 2 distal femur were increased. Thus, we were able to demonstrate using two different methods that early OA-cartilages show an increase in superficial chondrocytes suggesting that proliferation in the articular surface had occurred.

The presence of a novel organizational pattern in the superficial zone may also be related to chondrocyte death. In the present study, we focused on the spatial location of vital superficial chondrocytes or those that have recently died by cartilage dissection or donor death. We excluded structurally disintegrated chondrocytes that underwent apoptosis or necrosis previous to donor death since we stained the DNA of nuclei. We noted that almost all depicted nuclei were represented by a clear and spatially defined DNA signal. However, it is possible that chondrocytes that underwent apoptosis or necrosis previous to donor death contributed to the modified organization by causing acellular areas without any fluorescent staining or by interrupting the existing nuclei signals with focal absence of fluorescent stain. In that case, one would expect an overall decreased number of nuclei signals or, for example, shorter or interrupted condylar strings. However, the strings that we observed in non-degenerate condyles were not shorter or interrupted in the intact cartilage of grade 2 joints. In contrast, they were modified to double strings. Consequently, most cell groups in the intact cartilage of grade 2 joints contained more chondrocytes per group than in non-degenerate cartilage. We also showed an increase in the cell density of the superficial zone in the grade 2 distal femur compared to grade 0–1. Both observations suggest that an increased number of chondrocytes was present in the grade 2 distal femur. Thus, it is unlikely that the death of chondrocytes that died previously was responsible for the depicted organizational modification.

Another contributing factor relevant to the described organizational changes may be structural loss of the superficial zone in early OA. In that case, translucent nuclei fluorescent signals from the deeper zones may have mistakenly been analyzed as superficial zones.

However, we verified the presence of intact superficial zones by histological analyses. Thus, structural loss of the superficial zone unlikely contributed to the grade 2-specific modification of the horizontal chondrocyte organization. Furthermore, we showed that OA-typical cellular aggregates were never observed distant to lesions where the quantified spatial and numerical organizational changes occurred. Because we were unable to establish organizational patterns within these OA-typical cellular aggregates, we termed them ‘unorganized’. The ‘unorganized’ cellular aggregation did not contribute to the modification of the organizational patterns of superficial chondrocytes in early OA.

Often, stereological methods are applied to assess cell organization and density of articular cartilage (21,37,38). However, these studies did not identify the spatial and density-related cellular characteristics, which the present and our previous study unraveled (1). Therefore, we believe that the presently applied non-stereological methods were justified. Because it has been suggested that the yield of released cells from human cartilage is relatively low and perhaps depends on the amount of damage from slicing cartilage, we calculated the ratio between the densities of the superficial zone and the deeper zones and found that it was comparable to those of other studies (21–23).

Furthermore, comparisons of percentages of groups with different denominators must be interpreted cautiously because the percentage of cells in certain organizational patterns may be skewed by different total numbers of cells. Other metrics or a true 3-D study may be useful because a 3-D analysis showed recently that the nearest neighbor distance did not vary significantly with growth from the fetal to calf to adult stage of cartilage whereas the angle to the nearest neighboring cell was sensitive to changes in bovine chondrocyte organization (39). In contrast, the present and our previous study (1) showed that the metric “nearest neighbor distance” was sensitive enough to elucidate differences in the spatial organization of human superficial zone chondrocytes. Whereas the present study examined human cartilage, the previously mentioned study analyzed bovine tissue. The difference in the sensitivity of the metric “nearest neighbor distance” may partially be caused by species-derived differences. Nevertheless, it may have been advantageous for the present study to also assess the angle from a cell to its nearest neighbor.

In the current study, we demonstrated that human superficial chondrocytes underwent a remodeling of their organizational structure distant to local fibrillations or lesions. The remodeling process led to the formation of a novel organizational pattern across the intact articular surface suggesting a generalized and coordinated chondrocyte response to distant early OA. That chondrocytes are capable of remodeling their organizational structure was recently shown by a study, which assessed the organizational structure by the angle to the nearest neighboring cell. It was shown that the cell organization changed dramatically during growth, ultimately attaining the classical zonal organization of adulthood (39). The capacity of a generalized response is, however, not limited to chondrocytes during growth. A comparable ability of OA-chondrocytes was recently revealed by our laboratory when we demonstrated that the up-regulation of matrix turnover in ankle cartilage remote from OA lesions was similar to that adjacent to OA lesions (14). This up-regulation of matrix turnover was evident in ankle but not knee cartilage. However, the generalized upregulation of matrix-turnover suggested a coordinated response of the articular surface to repair the focally damaged matrix (14). Thus, superficial chondrocytes seem capable of coordinated responses.

The question as to the physiological and clinical relevance arises from the reported remodeling of the cellular organization in early OA. Our laboratory showed strong correlations between the spatial organizational chondrocyte patterns and the anatomical joint type in which they occurred (1). Those findings may suggest an association of the



organizational surface structure with local biomechanical or metabolic environments. Thus, changed local conditions in early OA may require the chondrocytes to adapt their spatial organization. However, it is not known how chondrocytes could benefit from an organizational remodeling, or whether it is actively sought out or a passive consequence of proliferation or synthesis. We believe that the increase in chondrocyte numbers may serve as recruitment of metabolically active units as an ultimately failing attempt to repair focal damage. A number of studies has shown that enhanced synthesis of extracellular matrix components is present in OA (40–48). The increase in cell numbers may suggest a not well understood regenerative potential of the remaining intact cartilage in early OA.

Studies on early OA are rare, partially because only a few laboratories have access to intact human donor tissues, whereas an abundance of studies on surgical end-stage OA specimens dominate the literature. However, in advanced OA, two studies analyzed the regenerative cellular potential; they showed that OA chondrocytes can generate type II collagen and proteoglycan-rich cartilage transplants (49). Although their bioactivity seemed lower (49,50), these advanced OA chondrocytes were considered suitable for autologous chondrocyte implantation (ACI) based on their mRNA expression patterns (49). Taken together, the present study implies clinical relevance because a regenerative potential may undoubtedly be desirable in tissue engineering and justifies further investigations of the capabilities of early OA chondrocytes. Further animal and human studies are currently being undertaken in our laboratory to elucidate the functional significance of the elucidated remodeling of the spatial organization of human superficial chondrocytes with early OA.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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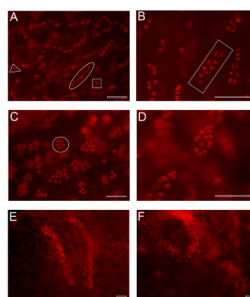
## References

1. Rolauffs B, Williams JM, Grodzinsky AJ, Kuettner KE, Cole AA. Distinct horizontal patterns in the spatial organization of superficial zone chondrocytes of human joints. *Journal of structural biology*. 2008; 162(2):335–44. [PubMed: 18325787]
2. Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. *Arthritis Res*. 2001; 3(2):107–13. [PubMed: 11178118]
3. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am*. 1971; 53(3):523–37. [PubMed: 5580011]
4. Brama PA, Tekoppele JM, Bank RA, Barneveld A, van Weeren PR. Functional adaptation of equine articular cartilage: the formation of regional biochemical characteristics up to age one year. *Equine Vet J*. 2000; 32(3):217–21. [PubMed: 10836476]
5. Maroudas A, Wachtel E, Grushko G, Katz EP, Weinberg P. The effect of osmotic and mechanical pressures on water partitioning in articular cartilage. *Biochim Biophys Acta*. 1991; 1073(2):285–94. [PubMed: 2009281]
6. Howell DS. Pathogenesis of osteoarthritis. *Am J Med*. 1986; 80(4B):24–8. [PubMed: 3010715]
7. Hamerman D. The biology of osteoarthritis. *N Engl J Med*. 1989; 320(20):1322–30. [PubMed: 2654632]

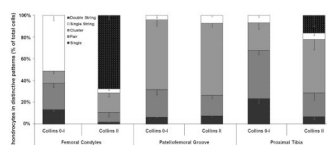
8. Vignon E, Arlot M, Meunier P, Vignon G. Quantitative histological changes in osteoarthritic hip cartilage. Morphometric analysis of 29 osteoarthritic and 26 normal human femoral heads. *Clin Orthop Relat Res.* 1974; (103):269–78. [PubMed: 4137904]
9. Mitchell N, Lee ER, Shepard N. The clones of osteoarthritic cartilage. *J Bone Joint Surg Br.* 1992; 74(1):33–8. [PubMed: 1732261]
10. Gardner DL, Salter DM, Oates K. Advances in the microscopy of osteoarthritis. *Microsc Res Tech.* 1997; 37(4):245–70. [PubMed: 9185149]
11. Schumacher BL, Su JL, Lindley KM, Kuettner KE, Cole AA. Horizontally oriented clusters of multiple chondrons in the superficial zone of ankle, but not knee articular cartilage. *Anat Rec.* 2002; 266(4):241–8. [PubMed: 11920387]
12. Weiss C, Mirow S. An ultrastructural study of osteoarthritis changes in the articular cartilage of human knees. *J Bone Joint Surg Am.* 1972; 54(5):954–72. [PubMed: 4262548]
13. Agha RA, Webb B. A cadaveric investigation into the links between macroscopic and microscopic osteoarthritic changes at the hip. *Clin Anat.* 2006; 19(2):115–24. [PubMed: 16283648]
14. Aurich M, Mwale F, Reiner A, Mollenhauer JA, Anders JO, Fuhrmann RA, et al. Collagen and proteoglycan turnover in focally damaged human ankle cartilage: evidence for a generalized response and active matrix remodeling across the entire joint surface. *Arthritis Rheum.* 2006; 54(1):244–52. [PubMed: 16388531]
15. Muehleman C, Bareither D, Huch K, Cole AA, Kuettner KE. Prevalence of degenerative morphological changes in the joints of the lower extremity. *Osteoarthritis Cartilage.* 1997; 5(1): 23–37. [PubMed: 9010876]
16. Rolauffs, B.; Chai, DH.; Muehleman, C.; Frank, EH.; Grodzinsky, AJ. Impairment of biomechanical properties occurs prior to GAG loss after injury and can be predicted by the injurious stress strain curve. 54th Annual Meeting of the Orthopaedic Research Society; 2008; San Francisco, CA, USA. 2008.
17. Diggle PJ, Mateu G, Clough HE. A comparison between parametric and non-parametric approaches to the analysis of replicated spatial point patterns. *Adv in Appl Probab.* 2000; 32(2): 331–43.
18. Rousseeuw PJ. Silhouettes: A Graphical Aid to the Interpretation and Validation of Cluster Analysis. *J Comput Appl Math.* 1987; (20):53–65.
19. Clark PJ, Evans FC. Distance to Nearest Neighbor as a Measure of Spatial Relationships in Populations. *Ecology.* 1954; 35(4):445–53.
20. Aydelotte MB, Kuettner KE. Differences between sub-populations of cultured bovine articular chondrocytes. I. Morphology and cartilage matrix production. *Connect Tissue Res.* 1988; 18(3): 205–22. [PubMed: 3219850]
21. Hunziker EB, Quinn TM, Hauselmann HJ. Quantitative structural organization of normal adult human articular cartilage. *Osteoarthritis Cartilage.* 2002; 10(7):564–72. [PubMed: 12127837]
22. Quinn TM, Hunziker EB, Hauselmann HJ. Variation of cell and matrix morphologies in articular cartilage among locations in the adult human knee. *Osteoarthritis Cartilage.* 2005; 13(8):672–8. [PubMed: 15970445]
23. Temple MM, Bae WC, Chen MQ, Lotz M, Amiel D, Coutts RD, et al. Age- and site-associated biomechanical weakening of human articular cartilage of the femoral condyle. *Osteoarthritis Cartilage.* 2007; 15(9):1042–52. [PubMed: 17468016]
24. Williams JM, Uebelhart D, Thonar EJ, Kocsis K, Modis L. Alteration and recovery of the spatial orientation of the collagen network of articular cartilage in adolescent rabbits following intra-articular chymopapain injection. *Connect Tissue Res.* 1996; 34(2):105–17. [PubMed: 8909875]
25. Meachim G, Collins DH. Cell counts of normal and osteoarthritic articular cartilage in relation to the uptake of sulphate ( $^{35}\text{SO}_4$ ) in vitro. *Ann Rheum Dis.* 1962; 21:45–50. [PubMed: 14471899]
26. Havdrup T, Telhag H. Mitosis of chondrocytes in normal adult joint cartilage. *Clin Orthop Relat Res.* 1980; (153):248–52. [PubMed: 7449224]
27. Hirotani H, Ito T. Chondrocyte mitosis in the articular cartilage of femoral heads with various diseases. *Acta Orthop Scand.* 1975; 46(6):979–86. [PubMed: 1211135]
28. Rothwell AG, Bentley G. Chondrocyte multiplication in osteoarthritic articular cartilage. *J Bone Joint Surg Br.* 1973; 55(3):588–94. [PubMed: 4729024]

29. Telhag H. Mitosis of chondrocytes in experimental "osteoarthritis" in rabbits. *Clin Orthop Relat Res.* 1972; 86:224–9. [PubMed: 5047793]
30. Telhag H. DNA-synthesis in degenerated and normal joint cartilage in full-grown rabbits. *Acta Orthop Scand.* 1973; 44(6):604–10. [PubMed: 4770129]
31. Hulth A, Lindberg L, Telhag H. Mitosis in human osteoarthritic cartilage. *Clin Orthop Relat Res.* 1972; 84:197–9. [PubMed: 5032842]
32. Muldrew K, Chung M, Novak K, Schachar NS, Zernicke RF, McGann LE, et al. Evidence of chondrocyte repopulation in adult ovine articular cartilage following cryoinjury and long-term transplantation. *Osteoarthritis Cartilage.* 2001; 9(5):432–9. [PubMed: 11467891]
33. Gomez-Camarillo MA, Kouri JB. Ontogeny of rat chondrocyte proliferation: studies in embryo, adult and osteoarthritic (OA) cartilage. *Cell research.* 2005; 15(2):99–104. [PubMed: 15740638]
34. Poole CA, Gilbert RT, Herbage D, Hartmann DJ. Immunolocalization of type IX collagen in normal and spontaneously osteoarthritic canine tibial cartilage and isolated chondrons. *Osteoarthritis Cartilage.* 1997; 5(3):191–204. [PubMed: 9219682]
35. Quintavalla J, Kumar C, Daouti S, Slosberg E, Uziel-Fusi S. Chondrocyte cluster formation in agarose cultures as a functional assay to identify genes expressed in osteoarthritis. *J Cell Physiol.* 2005; 204(2):560–6. [PubMed: 15799031]
36. Lee DA, Bentley G, Archer CW. The control of cell division in articular chondrocytes. *Osteoarthritis Cartilage.* 1993; 1(2):137–46. [PubMed: 8886090]
37. Egli PS, Hunziker EB, Schenk RK. Quantitation of structural features characterizing weight- and less-weight-bearing regions in articular cartilage: a stereological analysis of medial femoral condyles in young adult rabbits. *Anat Rec.* 1988; 222(3):217–27. [PubMed: 3213972]
38. Wong M, Wuethrich P, Egli P, Hunziker E. Zone-specific cell biosynthetic activity in mature bovine articular cartilage: a new method using confocal microscopic stereology and quantitative autoradiography. *J Orthop Res.* 1996; 14(3):424–32. [PubMed: 8676256]
39. Jadin KD, Bae WC, Schumacher BL, Sah RL. Three-dimensional (3-D) imaging of chondrocytes in articular cartilage: growth-associated changes in cell organization. *Biomaterials.* 2007; 28(2): 230–9. [PubMed: 16999994]
40. Lippiello L, Hall D, Mankin HJ. Collagen synthesis in normal and osteoarthritic human cartilage. *J Clin Invest.* 1977; 59(4):593–600. [PubMed: 845251]
41. Eyre DR, McDevitt CA, Billingham ME, Muir H. Biosynthesis of collagen and other matrix proteins by articular cartilage in experimental osteoarthrosis. *Biochem J.* 1980; 188(3):823–37. [PubMed: 7470037]
42. Collins DH, Mc ET. Sulphate (35SO<sub>4</sub>) uptake by chondrocytes in relation to histological changes in osteoarthritic human articular cartilage. *Ann Rheum Dis.* 1960; 19:318–30. [PubMed: 13694746]
43. McDevitt CA, Muir H. Biochemical changes in the cartilage of the knee in experimental and natural osteoarthritis in the dog. *J Bone Joint Surg Br.* 1976; 58(1):94–101. [PubMed: 131804]
44. Mankin HJ, Johnson ME, Lippiello L. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. III. Distribution and metabolism of amino sugar-containing macromolecules. *J Bone Joint Surg Am.* 1981; 63(1):131–9. [PubMed: 7451514]
45. Mitrovic D, Gruson M, Demignon J, Mercier P, Aprile F, De Seze S. Metabolism of human femoral head cartilage in osteoarthrosis and subcapital fracture. *Ann Rheum Dis.* 1981; 40(1):18–26. [PubMed: 7469522]
46. Ryu J, Treadwell BV, Mankin HJ. Biochemical and metabolic abnormalities in normal and osteoarthritic human articular cartilage. *Arthritis Rheum.* 1984; 27(1):49–57. [PubMed: 6691859]
47. Sandy JD, Adams ME, Billingham ME, Plaas A, Muir H. In vivo and in vitro stimulation of chondrocyte biosynthetic activity in early experimental osteoarthritis. *Arthritis Rheum.* 1984; 27(4):388–97. [PubMed: 6712755]
48. Aigner T, Stoss H, Weseloh G, Zeiler G, von der Mark K. Activation of collagen type II expression in osteoarthritic and rheumatoid cartilage. *Virchows Arch B Cell Pathol Incl Mol Pathol.* 1992; 62(6):337–45. [PubMed: 1280884]

49. Stoop R, Albrecht D, Gaissmaier C, Fritz J, Felka T, Rudert M, et al. Comparison of marker gene expression in chondrocytes from patients receiving autologous chondrocyte transplantation versus osteoarthritis patients. *Arthritis Res Ther.* 2007; 9(3):R60. [PubMed: 17596264]
50. Dorotka R, Bindreiter U, Vavken P, Nehrer S. Behavior of human articular chondrocytes derived from nonarthritic and osteoarthritic cartilage in a collagen matrix. *Tissue Eng.* 2005; 11(5–6):877–86. [PubMed: 15998227]

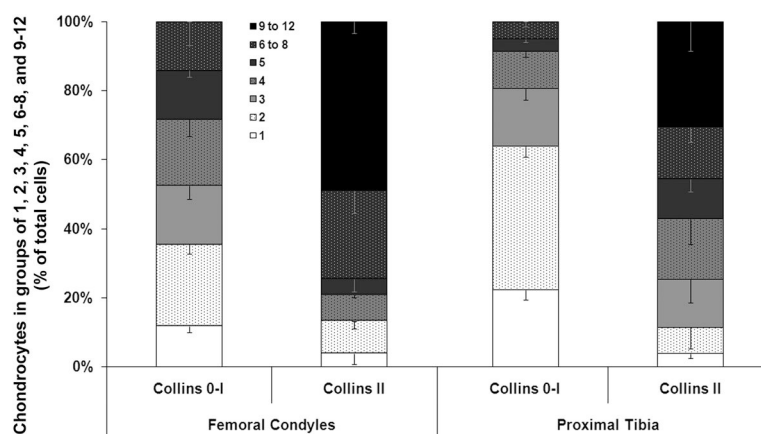


**Figure 1. Top-down view onto the articular surface depicting the horizontally orientated organizational patterns of the superficial zone chondrocytes with and without early OA**  
Fluorescent microscopic representative images showing chondrocyte nuclei stained with propidium iodide of the femoral condyles (A-B, E-F), and the patellofemoral groove (C-D). Shown is the normal, non-osteoarthritic articular surface of grade 0–1 joints (A, C), the remaining, intact articular surface distant to a focal OA lesion of grade 2 joints (B, D), and a focal lesion of the articular surface of grade 2 condyles (E, F). Square: single chondrocyte; triangle: pair; circle: cluster; ellipse: string; rectangle: double string. Scale bars, 100  $\mu$ m.



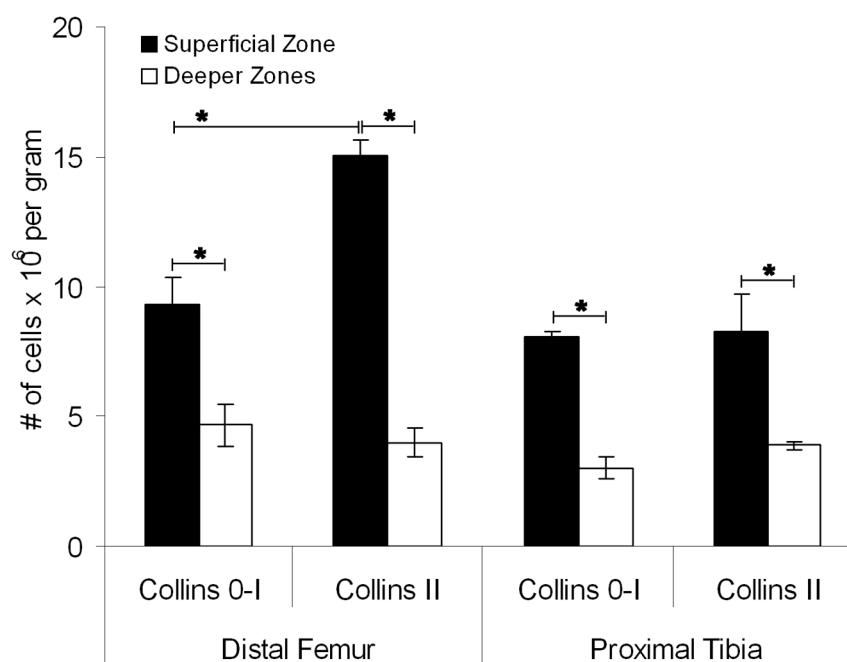
**Figure 2. Percentage of chondrocytes that were grouped in strings, clusters, pairs or as singles**  
Values are expressed as the mean  $\pm$  standard error of the mean (SEM). *p* values are listed in Table I. Strings, clusters, pairs and singles are shown in Fig. 1.





**Figure 3. Percentage of chondrocytes that appear as single cells or in groups of 2, 3, 4, 5, 6 to 8, or 9 to 12 cells**

Values are expressed as the mean  $\pm$  SEM.



**Figure 4. Cell densities of the superficial and the deeper zones of the human knee joint**  
 Values are expressed as the mean  $\pm$  SEM. \*,  $p < 0.05$ . The cell density of the distal femur consisted of the groove and the condylar regions; separate densities for both regions were not calculated.

**Table I**

Significant differences between articular surfaces and OA grades

Pattern	Comparison	Critical <i>p</i> Level	Significance
<b>Strings</b>	Condyles grade 0–1 vs. Condyles grade 2	0.001	Yes
	PFG grade 0–1 vs. PFG grade 2	0.05	No
	Condyles grade 0–1 vs. PFG grade 0–1	0.001	Yes
	Condyles grade 2 vs. PFG grade 2	0.05	No
	Prox Tibia grade 0–1 vs. Prox. Tibia grade 2	0.05	No
	Prox Tibia grade 2 vs. Condyles grade 2	0.05	No
	Prox Tibia grade 2 vs. PFG grade 2	0.05	No
<b>Double strings</b>	Condyles grade 0–1 vs. Condyles grade 2	0.05	Yes
	PFG grade 0–1 vs. PDF grade 2	0.05	No
	Condyles grade 0–1 vs. PFG grade 0–1	0.05	No
	Condyles grade 2 vs. PFG grade 2	0.05	Yes
	Prox Tibia grade 0–1 vs. Prox. Tibia grade 2	0.05	No
	Prox Tibia grade 2 vs. Condyles grade 2	0.05	No
	Prox Tibia grade 2 vs. PFG grade 2	0.05	No
<b>Cluster</b>	Condyles grade 0–1 vs. Condyles grade 2	0.05	No
	PFG grade 0–1 vs. PFG grade 2	0.05	No
	Condyles grade 0–1 vs. PFG grade 0–1	0.001	Yes
	Condyles grade 2 vs. PFG grade 2	0.001	Yes
	Prox Tibia grade 0–1 vs. Prox. Tibia grade 2	0.05	Yes
	Prox Tibia grade 2 vs. Condyles grade 2	0.05	No
	Prox Tibia grade 2 vs. PFG grade 2	0.05	No
<b>Pairs</b>	Condyles grade 0–1 vs. Condyles grade 2	0.05	No
	PFG grade 0–1 vs. PDF grade 2	0.05	No
	Condyles grade 0–1 vs. PFG grade 0–1	0.05	No
	Condyles grade 2 vs. PFG grade 2	0.05	No
	Prox Tibia grade 0–1 vs. Prox. Tibia grade 2	0.01	Yes
	Prox Tibia grade 2 vs. Condyles grade 2	0.05	No
	Prox Tibia grade 2 vs. PFG grade 2	0.05	No
<b>Singles</b>	Condyles grade 0–1 vs. Condyles grade 2	0.01	Yes
	PFG grade 0–1 vs. PDF grade 2	0.05	No
	Condyles grade 0–1 vs. PFG grade 0–1	0.05	No
	Condyles grade 2 vs. PFG grade 2	0.05	No
	Prox Tibia grade 0–1 vs. Prox. Tibia grade 2	0.001	Yes
	Prox Tibia grade 2 vs. Condyles grade 2	0.05	No
	Prox Tibia grade 2 vs. PFG grade 2	0.05	No