Purpose: Cell cluster formation in articular cartilage is a hallmark in osteoarthritis (OA). These cell clusters have been associated with both cartilage catabolism and anabolism. We addressed this issue by investigating the effect of clustering chondrocytes in highly controlled micro-aggregates on their morphology, stability and chondrogenic potential.

Methods: We designed a micro-mold that enables controlled formation of micro-aggregates ranging from 20 to 200 cells in high throughput. Morphology, stability and chondrogenic potential of micro-aggregates was evaluated and compared to single-cells cultured in micro-wells and in 3D after encapsulation in a Dextran-Tyramine (Dex-TA) hydrogel in vitro and in vivo after subcutaneous implantation in mice.

Results: We successfully formed micro-aggregates with highly controlled size, morphology, cell density, stability and viability. Micro-aggregates of 100 cells presented a superior balance in Collagen type I and Collagen type II gene expression over micro-aggregates of 20 and 50 cells. Matrix metalloproteinases (1, 9 and 13) gene expression was decreased in micro-aggregates compared to single cells. Histological analysis of hydrogels cultured in vitro and after implantation in mice demonstrated enhanced matrix deposition in constructs seeded with micro-aggregates, compared to single-cell seeded constructs.

Conclusions: Using a highly controlled model for high throughput formation of micro-aggregates, we demonstrated that clustering chondrocytes in micro-aggregates stimulated cartilage matrix formation. Consequently, the use of micro-aggregates, compared to single cells, resulted in greatly improved neo-cartilage formation. Furthermore, our data provided experimental evidence suggesting that cartilage clusters found in osteoarthritic cartilage are part of a regenerative response.

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EXPRESSION OF P53R2 IN CHONDROCYTES IS REGULATED BY MECHANICAL STRESS


Purpose: p53 tumor suppressor protein is activated in response to DNA damage. Ser46 residue of p53 is phosphorylated and p53AP1 (apoptosis inducing protein) induced apoptosis in case of severe DNA damage. While the damage is not so severe, Ser15 residue of p53 is phosphorylated and p53R2 repaired DNA damage. We previously showed that excessive mechanical stress induced chondrocytes apoptosis via p53 and p53AP1 pathway. In this study, we evaluated p53R2 expression and function of chondrocytes in response to mechanical stress.

Methods: OA cartilage samples were obtained from total knee replacement surgery, and normal cartilage samples were from femoral neck fracture. The expression of p53R2 was analyzed by immunohistochemistry. Chondrocytes were isolated from OA and normal cartilage. The expression of p53R2 in chondrocytes was detected by western blotting and real-time PCR. OA chondrocytes were introduced 2, 5 and 10% tensile strain for 12hours by using FX-2000. After the strain, expressions of p53R2, type 2 collagen and aggrecan were detected by real-time PCR. The phosphorylation of Ser15 and Ser46 residue of p53 was analyzed by western blotting. p53R2 siRNA was transfected to OA chondrocytes, and expressions of type2 collagen and aggrecan were detected after tensile strain.

Results: p53R2 was highly expressed in OA cartilage in comparison with normal cartilage by immunohistochemistry. Western blotting and real-time PCR showed p53R2 expression in chondrocytes was higher in OA chondrocytes than in normal chondrocytes. p53R2 expression in OA chondrocytes was increased after 2 and 5% tensile strain and decreased after 10% tensile strain. p53R2, Type2 collagen and aggrecan expressions were increased after 2 and 5% tensile strain but decreased after 10% strain. Ser15 residue of p53 was phosphorylated after 5% strain, but Ser46 residue was not. After the transfection of p53R2 siRNA, expressions of type2 collagen and aggrecan were down-regulated.

Conclusions: In our study, p53R2 in chondrocytes was increased after 2 and 5% tensile strain. Down-regulation of p53R2 reduced type 2 collagen and aggrecan expression via Ser15 of p53 in response to mechanical stress. Up-regulation of p53R2 may increase type 2 collagen and aggrecan expression via Ser15 of p53 in response to mechanical stress. We consider that regulation of p53R2 might be one of strategy for OA treatment. We are doing further investigation to analyze p53R2 function in chondrocytes.

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CHONDROCYTE CLUSTER FORMATION STIMULATES CARTILAGE MATRIX DEPOSITION

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Purpose: Cluster formation in articular cartilage is a hallmark in osteoarthritis (OA). These cell clusters have been associated with both cartilage catabolism and anabolism. We addressed this issue by investigating the effect of clustering chondrocytes in highly controlled micro-aggregates on their morphology, stability and chondrogenic potential.

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FEASIBILITY OF CHONDROCYTE CULTURES FROM CADAVER FINGER JOINTS

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Purpose: Chondrocyte cultures are instrumental for studying the pathogenesis of osteoarthritis of the hands. We investigated the feasibility of using human cartilage from fingers of dissecting room cadavers donated to the institute of anatomy.

Methods: Proximal interphalangeal joints (PIP) were obtained from 17 untreated dissecting room cadavers (mean age 66) and a post mortem time up to 101 hours. The joint surface appeared macroscopically osteoarthritic in four cases. Cartilage and connective tissue was harvested under sterile conditions. As a control we used cartilage derived from patients undergoing total knee joint replacement. Tissues were digested in collagenase B and cultured in Ham's F-12/DMEM (1:1) and 10% FBS over 3 passages. Gene expression of matrix metalloprotease (MMP)-13, inducible nitric oxide synthase (iNOS), Collagen II and X and alkaline phosphatase was evaluated using quantitative real-time PCR and western blot.

Results: Chondrocytes from cadavers up to 101 hours post mortem were viable in all cases. Cell yields were comparable to controls with an average of 3*10^6 cells after two cell culture passages. RNA was isolated with an average of 500 ng to 1000 ng. Chondrocytes from PIP exhibited typical morphological and expression of Collagen II when compared to fibroblasts. In comparison with articular chondrocytes from patients undergoing knee joint replacement cultured chondrocytes from PIP had significantly lower basal expression of iNOS (48 fold, P<0.01) and Collagen X (3.7 fold, P<0.01). In contrast basal gene expression of alkaline phosphatase were found to be elevated 15 fold (P<0.01) on hand OA chondrocytes. Expression of MMP-13 was not significantly different.

Conclusions: Cadaver chondrocyte culture from finger joints is feasible. In this pilot study, chondrocytes from hand osteoarthritis showed differences in the expression of iNOS and collagen genes.

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AGING AND OXIDATIVE STRESS REDUCE THE CHONDROCYTE RESPONSE TO IGF-1 AND OP-1

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Purpose: The mechanisms by which aging changes in the joint predispose older adults to develop OA are incompletely understood. Oxidative stress is thought to play a role in age-related conditions. The purpose of this study was to determine the effects of age and oxidative stress on the chondrocyte response to IGF-1 and OP-1, two important anabolic factors in cartilage.

Methods: Human articular chondrocytes were isolated from normal talar cartilage obtained from over 40 tissue donors with ages ranging from 19–91yrs. Cells in confluent primary cultures or in alginate beads were made serum-free and stimulated with 50–100 ng/ml IGF-1, OP-1, or