degradation. MES in combination with HS can be a therapeutic stimuli for synovial joints by inducing HSP70 in articular cartilage.

Method: Human S chondrocytes from the macroscopically normal parts of 3 osteoarthritic joints (P2) were encapsulated in 2% alginate and pre-cultured in serum-free chordogenic media supplemented with TGF-β1 for 2 weeks. Following pre-culture, constructs were dynamically compressed (1 Hz, 50% strain, 3hr) for 2 weeks. For the analysis of MMPs, conditioned media were collected at each media change. Concentrations of MMP-3 and MMP-13 were measured using a multiplex ELISA (Millipore). After 2 weeks of compression, S constructs were embedded in paraffin for immunofluorescence staining of collagen types II, IV, VI, and IX.

Results: Dynamic compression reduced MMP production by S chondrocytes compared to the controls with no loading. Without compressive stimulation, S chondrocytes on average produced higher levels of MMP-3 (~250-fold) and MMP-13 (~2.4-fold). Immunofluorescence images also showed brighter staining of collagen types II, IV, VI, and IX in S constructs subjected to compression compared to those with no loading.

Conclusions: Mechanical load is a potent modulator of chondrocyte expression of MMPs, and hence cartilage homeostasis. While injurious loading can initiate cartilage degradation and OA, absence of mechanical stimulation can also result in elevated levels of MMPs, cartilage thinning and proteoglycan loss in vivo. Our in vitro model was able to detect major decreases in MMP expression by S chondrocytes from OA patients with moderate in vitro loading. These data highlight the importance of appropriate mechanical stimulation in maintaining cartilage matrix, and also implicate loading as an important parameter to optimize when engineering articular cartilage from OA chondrocytes.

224 EXPRESSION PROFILE OF CARBONIC ANHYDRASES IN ARTICULAR CARTILAGE
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Purpose: Carbonic anhydrases (CAs), which catalyze the reversible reaction of carbonate hydration, are important for cartilage homeostasis. The full spectrum of CA activity of all 13 isoenzymes in articular cartilage is unknown.

Methods: This study quantified an expression profile of CAs in rat articular cartilage, using quantitative polymerase chain reactions, and localized the CAs that were significantly expressed by chondrocytes in the zonal structure of cartilage using immunohistochemistry.

Results: Among the 13 functional CAs, CAs II, III, Vb, IX, XII and XIII were significantly expressed in articular cartilage. The expression of CA III spanned across the full thickness of articular cartilage. CA IX was limited in the superficial zone of cartilage and CA XIII expressed in the superficial and partially mid zone. CA II was seen in the mid and deep zone. CA XII was more restricted in the deep zone and CA Vb was found in the deep zone and subchondral bone.

Conclusion: Since CAs play a role in mineralization and demineralization, these results provide a framework for understanding individual CAs as well as the integrated CA family in cartilage biology and pathology.

225 SUPERFICIAL CHONDROCYTES FROM OSTEOARTHRITIS PATIENTS HAVE REDUCED MATRIX METALLOPROTEINASE EXPRESSION FOLLOWING COMPRESSION
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Purpose: Articular cartilage functions as a shock absorber in diarthrodial joints. During joint loading, the superficial (S) zone of the cartilage can be subjected to compressive strains of up to 50%. When damaged, cartilage degeneration occurs, eventually leading to osteoarthritis (OA). Cartilage degradation usually starts from the superficial zone, reaching the deeper zones as the disease progresses. Matrix metalloproteinases (MMPs) are collagenases that are up-regulated following cartilage injury and play a role in matrix degradation and OA.

We hypothesize that S chondrocytes from macroscopically normal parts of the OA joints can be used to model early-stage OA, and that loading will affect the production of MMPs. In this study, we investigated the effects of dynamic compression on S chondrocytes obtained from OA patients, and its influence in modulating MMP production.

Method: Human S chondrocytes from the macroscopically normal parts of 3 osteoarthritic joints (P2) were encapsulated in 2% alginate and pre-cultured in serum-free chordogenic media supplemented with TGF-β1 for 2 weeks. Following pre-culture, constructs were dynamically compressed (1 Hz, 50% strain, 3hr) for 2 weeks. For the analysis of MMPs, conditioned media were collected at each media change. Concentrations of MMP-3 and MMP-13 were measured using a multiplex ELISA (Millipore). After 2 weeks of compression, S constructs were embedded in paraffin for immunofluorescence staining of collagen types II, IV, VI, and IX.

Results: Dynamic compression reduced MMP production by S chondrocytes compared to the controls with no loading. Without compressive stimulation, S chondrocytes on average produced higher levels of MMP-3 (~250-fold) and MMP-13 (~2.4-fold). Immunofluorescence images also showed brighter staining of collagen types II, IV, VI, and IX in S constructs subjected to compression compared to those with no loading.

Conclusions: Mechanical load is a potent modulator of chondrocyte expression of MMPs, and hence cartilage homeostasis. While injurious loading can initiate cartilage degradation and OA, absence of mechanical stimulation can also result in elevated levels of MMPs, cartilage thinning and proteoglycan loss in vivo. Our in vitro model was able to detect major decreases in MMP expression by S chondrocytes from OA patients with moderate in vitro loading. These data highlight the importance of appropriate mechanical stimulation in maintaining cartilage matrix, and also implicate loading as an important parameter to optimize when engineering articular cartilage from OA chondrocytes.

226 ANTI-FIBROTIC EFFECT OF ADIPOSE STROMAL CELLS IN COCULTURE WITH CHONDROCYTES FROM OSTEOARTHRITHIC PATIENTS
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Purpose: Osteoarthritis (OA) characterized by degeneration of articular cartilage is the most frequent rheumatic disease. Mesenchymal stem cells (MSC) isolated from bone marrow (MSC) or adipose tissues (adipose stromal cells (ASC)) secrete a large amount of factors (angiogenic, proliferative, anti-fibrotic or anti-apoptotic properties. The possibility that these cells, through their trophic potential, may influence the course of chronic degenerative disorders and prevent cartilage degradation is promising for the treatment of OA. The aim of our work was to evaluate the effects of ASC or MSC on OA chondrocyte phenotype in vitro.

Methods: OA ASC were isolated from intra-articular (Hoffa-ASC) or hip (hip ASC) subcutaneous adipose tissue and healthy ASC from abdominal depot (abdo-ASC). MSC were obtained from healthy and OA donors. ASC or MSC were co-incubated with OA chondrocytes cultured either in monolayer or in pellet during 2 or 7 days using cell culture inserts. We evaluated the specific markers of mature chondrocytes collagen II (Coll II), aggregan (Agg), link and sox9). Hypertrophic chondrocytes (MMP13, collagen X and alkaline phosphatase (AP)) and fibroblasts (collagen I and III) by RT-qPCR analysis. Secreted factors were quantified by ELISA.

Results: After 2 or 7 days, chondrocytes co-cultured in pellet with abdo-ASCs exhibited no change in the expression level of the markers tested. On the contrary, in monolayer, abdo-ASC induced a significant decrease of collagen II (Coll II), MMP13 and Coll I expression in chondrocytes at D2. After 7 days, we observed a stable expression of the markers specific for mature chondrocyte and a diminution of MMP13, AP, Coll I and Coll III. Compared to abdo-ASCs, Hoffa-ASC and Hip-ASC behaved differently. They reduced both mature chondrocyte makers and hypertrophic/fibrosis markers. When comparing MSC from healthy subjects, we measured a decrease of Agg, Link and Sox9 expression and stable levels of hypertrophic/fibrotic markers in OA chondrocytes whereas MSC from OA patients maintain chondrocyte marker expression and reduce hypertrophic/fibrotic markers (MMP13, Coll I and Coll III). Finally, factors known to be involved in fibrosis and matrix remodeling (HGF, TIMP-1 and -2, MMP-1 and -9, IL1-RA, IL1β...