Results: BAP level was higher in OA synovial fluid than in simple synovitis, and d-ROM level was slightness lower in OA than control synovitis, but these differences were not significant. BAP and d-ROM levels were not related with OA grade.

After intra-articular injection of HA, BAP level was significantly increased and d-ROM level was reduced. On the other hand, BAP and d-ROM level were significantly decreased after injection of corticosteroid.

Conclusions: Oxidative stress and anti-oxidative potential in synovial fluid were not significant difference between OA and simple synovitis. Our results showed that hyaluronan increase the anti-oxidant potential fluid were not significant difference between OA and simple synovitis.

For statistical analysis, univariate analysis of variance was performed. To parameters, glycosaminoglycan (GAG) release and content, safranin-O staining and PGE2 production were determined.

222

INHIBITION OF PROSTAGLANDIN E2 BY CELECOXIB DECREASES GLYCOSAMINOGLYCAN RELEASE, HOWEVER DOES NOT STIMULATE REPAIR OF OSTEOARTHRITIC CARTILAGE TISSUE

M. Beehuizen1, A.I. Tsuchida2, A.G. Bot1, D.B. Saris1, W.J. Dhert1, L.B. Creemers1, G.J. van Osch1, 1UMC Utrecht, Utrecht, Netherlands; 2Erasmus MC, Rotterdam, Netherlands

Purpose: The pro-inflammatory lipid mediator prostaglandin E2 (PGE2) is present in the osteoarthritic joint and is know to play a role in osteoarthritis (OA). Both stimulated chondrocytes and synovial fibroblasts are capable of producing PGE2, however, to which extent osteoarthritic joint tissue is involved in its production and if the PGE2 produced has an effect on cartilage degeneration and regeneration is not entirely clear. Recently, we set up a coculture model with OA cartilage and OA synovial tissue that demonstrated to be more representative for OA than cartilage explant monocolonies. To further clarify the role of PGE2 in osteoarthritis, the effect of blocking COX-2 and hence production of PGE2, on cartilage degeneration and cartilage regeneration was studied in the coculture model and in a chondrocyte 3D regeneration model, respectively.

Methods: OA cartilage and OA synovial tissue were cultured alone or in coculture for 21 days with the addition of celecoxib at 0.1, 1 and 10 µM. Cartilage regeneration in the presence of absence of 1 µM celecoxib, was studied by seeding healthy or osteoarthritic chondrocytes (P2) at high density on collagen-coated filters and cultured for 28 days. As outcome parameters, glycosaminoglycan (GAG) release and content, safranin-O staining and PGE2 production were determined.

For statistical analysis, univariate analysis of variance was performed. To correct for inter-donor variability a randomized block design was used. P values <0.05 were considered significant.

Results: Both osteoarthritic cartilage and synovial tissue produced PGE2, albeit at much higher amounts by synovial tissue. Celecoxib inhibited PGE2 production (Fig. 1) and at 10 µM decreased glycosaminoglycan (GAG) release in coculture (Fig. 1), however, had no effect on GAG content in either cartilage monocolonore or coculture with OA synovial tissue (Fig. 1).

During regeneration, OA chondrocytes produced low amounts of PGE2, which also could be inhibited by celecoxib (Fig 2; P <0.05). However, celecoxib had no effect on cartilage regeneration in either the healthy and osteoarthritic chondrocytes (Fig. 2).

Conclusions: Osteoarthritic cartilage explants, OA chondrocytes and in particular OA synovial tissue produced PGE2, which could be inhibited by celecoxib, suggesting COX-2 was the main COX active in the tissue. In coculture, celecoxib inhibited both PGE2 production and GAG release, although no effect on GAG content was observed. During cartilage regeneration, the decrease of the PGE2 production was not accompanied by an effect on chondrocyte metabolism. This study shows that celecoxib inhibits PGE2 production in both explant and regeneration culture, however, its effect on cartilage metabolism is limited.

Fig. 1. PGE2 production, GAG release and GAG content in a coculture model with OA cartilage and synovial tissue.

Fig. 2. Celecoxib decreases PGE2 production in OA chondrocytes (*P <0.05), however without effect on GAG content.

223

MILD ELECTRICAL STIMULATION WITH HEAT SHOCK INCREASES HEAT SHOCK PROTEIN 70 AND PROTEOGLYCAN CORE PROTEIN IN ARTICULAR CARTILAGE

N. Hiraoka1, Y. Arai1, K.A. Takahashi2, R. Terauchi1, A. Inoue1,2, S. Tsuchida1, H. Inoue1, T. Kido1, 1Dept. of Orthopaedics, Graduate Sch. of Med. Sci., Kyoto Prefectural Univ. of Med., Kyoto, Japan; 2Dept. of Orthopaedics, Kyoto First Red Cross Hosp., Kyoto, Japan; 2Dept. of Rheumatology, Nippon Med. Sch., Tokyo, Japan

Purpose: Heat shock protein 70 (HSP70) inhibits the apoptosis of chondrocytes and has the protective effect on the cartilage. Meanwhile, we reported that mild electrical stimulation (MES) increased HSP70 by attenuating proteasomal degradation and that heat shock (HS) accelerated the translation of HSP70 in articular chondrocytes. The purpose of this study is to investigate the effect of MES in combination with heat shock (HS) on HSP70 in articular cartilage.

Methods: Sprague-Dawley rats (8 weeks of age, 200 g) were divided into 4 groups: Control group, MES group, HS group and HS+MES group. Rats were anesthetized and electrical stimulation was delivered to their left knee through a pair of 2.5 cm diameter electro-conductive and thermogenarative rubber electrodes (45°C) for 30 minutes. The electrodes were connected to a Biometronome (Tschiya Gum Co., Ltd., Kumamoto, Japan) that delivered 12 V (55 pps) of direct current with individual pulse duration of 0.1 ms. For the control group, rats were sham treated as above for 30 minutes per session but without conducting HS+MES. Eight hours after the treatment, protein lysates were obtained from cartilage fragments and subjected to Western blotting for HSP70 and ubiquitinated proteins (n = 4 each). Total RNA was isolated from the cartilage 24 hours after the HS+MES treatment. Relative expressions of mRNA for HSP70 and proteoglycan core protein (PG) were examined by real-time PCR (n = 6 each).

Results: After the treatment, we cannot find any adverse effect such as joint injury or gait disorder. Western blotting showed that HS modestly augmented HSP70. We found a remarkable increase of HSP70 and ubiquitinated proteins in HS+MES treated rats compared with the other groups (Figure 1). Real-time PCR analysis proved that MES did not affect the HSP70 mRNA. Compared to the control group, the HSP70 mRNA expression increased in the HS group and HS+MES group, and there was no significant difference between these two groups (Figure 2A). The expression of PG mRNA was significantly enhanced in the HS+MES group (Figure 2B).

Conclusions: In this study, HSP70 and PG mRNA in articular cartilage significantly increased by MES in combination with HS. HSPs constitute a family of highly conserved proteins which are synthesized in cells after stress loading including heat stress. HSP70 not only protect cells from various forms of stress, but also facilitate the recovery from stress-induced cell injury as molecular chaperones. We have reported that adenoviral overexpression of HSP70 resulted in promotion of PG transcription in chondrocytes, protection of chondrocytes from heat stress, and inhibition of NO-induced apoptosis of chondrocytes. Moreover, plasmid delivery of HSP70 in rat patellar cartilage decreased the severity of osteoarthritis-lesions. In the HS+MES group, PG mRNA could be induced by the HSP70. From the clinical point of view, effective and less-invasive method of HSP70 induction in articular chondrocyte could contribute to the treatment of osteoarthritis. MES and HS have already used as the safety method of HSP70 induction in vivo and attenuates hepatic ischemia/reperfusion injury. It is reported that MES induce HSP70 and ubiquitinated proteins via attenuation of proteosomal degradation. The elevation of HSP70 and ubiquitinated proteins in articular cartilage could be also due to the attenuation of proteosomal degradation.
degradation. MES in combination with HS can be a therapeutic stimuli for synovial joints by inducing HSP70 in articular cartilage.

Ubiquitinated protein
HSP70
actin
HS
MES
– – + +

Fig. 1.

**224**

**EXPRESSION PROFILE OF CARBONIC ANHYDRASES IN ARTICULAR CARTILAGE**


**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 XII 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**

J.E. Jeon. Queensland Univ. of Technology, Kelvin Grove, Australia

**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 chondrogenic 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.

**Cartilage Repair and Mesenchymal Cells**

**226**

**ANTI-FIBROTIC EFFECT OF ADIPOSE STROMAL CELLS IN COCULTURE WITH CHONDROCYTES FROM OSTEOARTHRITIC PATIENTS**

M. Maumus1, C. Manfredini1, K. Toupet1, J-A. Peyrafitte1, A. Piacentini2, E. Gabusi2, A. Facchini2, P. Bourn1, C. Jorgensen2, G. Lisignoli1, D. Noel.1 Inserm, Montpellier, France; 2IOR, Bologna, Italy; 1EFS-PM, Toulouse, France

**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 with immunomodulatory, 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 IIB (col IIB), 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-ASC exhibited no change in the expression level of the markers tested. On the contrary, in monolayer, abdo-ASC induced a significant decrease of col IIB, MMP13 and col 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, Col I and Col 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, Col I and Col III). Finally, factors known to be involved in fibrosis and matrix remodeling (HGF, TIMP-1 and -2, MMP-1 and -9, IL1-RA, IL1β

**227**

**AGING CHONDROCYTES IN IN VITRO ARE PRO-METABOLICALLY ACTIVATED AND PRO-INFLAMMATORY**

S. Alli, D. Shani, G. Stoica, M. Afshar, C. Ahn, K. Diao, P. Hovius, N. Doherty. Univ. of Pittsburgh, Pittsburgh, PA, USA

**Purpose:** Aging cartilage is increasingly recognized as an important risk factor for OA. Although cartilage is known to undergo a striking metabolic shift as a function of age, little is known about the specific metabolic pathways that contribute to the pro-inflammatory phenotype of elderly cartilage.

**Methods:** Human articular cartilage was obtained from patients undergoing total knee replacement due to OA. The cells were isolated and cultured in vitro for 28 days. We assessed the expression of metabolic pathways and pro-inflammatory markers using RT-qPCR and ELISA analysis, respectively.

**Results:** Aging chondrocytes showed a significant increase in the expression of genes associated with the pro-metabolic phenotype, including pyruvate dehydrogenase (PDK4) and acetyl-CoA carboxylase (ACC), as well as a concomitant increase in the expression of pro-inflammatory markers, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α).

**Conclusions:** Aging chondrocytes exhibit a pro-metabolic phenotype that may contribute to the pro-inflammatory state observed in OA. These findings suggest potential targets for therapeutic interventions aimed at reducing inflammation and metabolic dysfunction in aging cartilage.

**228**

**INFLAMMATORY RESPONSE OF BONE MARROW DERIVED STEM CELLS TO HYALURONIC ACID AND HUMAN SERUM**


**Purpose:** Bone marrow-derived stem cells (BMSCs) are a promising source for the treatment of OA. However, the inflammatory response of BMSCs to extracellular matrix components, such as hyaluronic acid (HA) and human serum, is not well understood.

**Methods:** Human BMSCs were cultured in the presence of HA and human serum, and their inflammatory response was assessed using RT-qPCR and ELISA analysis. The expression of inflammatory markers and cytokines was measured.

**Results:** BMSCs showed a significant increase in the expression of inflammatory markers, including interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), when cultured in the presence of HA and human serum.

**Conclusions:** The inflammatory response of BMSCs to HA and human serum is a potential mechanism for the development of OA. Understanding this response may provide insights into the development of therapeutic strategies to manage inflammatory processes in OA.