each condition were performed on 4 test/control pairs for both cB and cAC. To verify that the cB removed a significant amount of the DS, specimens were cut into 14 sections, 7 in a top half layer and 7 in a bottom half layer, and Western blot analysis for the decorin protein performed on each section. For comparison, cAC specimens were cut in a similar fashion and total proteoglycans assayed with the DMB assay.

**Results:** cB had no detectable effect on rapid modulus, equilibrium modulus, or relaxation function in indentation. Conversely, cAC caused reduction in equilibrium modulus and relaxation function, consistent with published work of others. Approximately one-half of the DS was removed from the top layer. Assay of the cAC specimens for total proteoglycan showed similar digestion patterns, suggesting that the two enzymes acted similarly.

**Conclusions:** Removal of DS from the cartilage did not affect the viscoelastic properties of the cartilage, indicating that decorin is not a structural molecule in cartilage in indentation.

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**P202**

**ACTIVATION OF C-JUN N-TERMINAL KINASE (JNK) IN ARTICULAR CHONDROCYTES BY MERCURY AND LEAD**

J.B. Collins, J.W. Neal, R.D. Graff

UNC School of Medicine, Chapel Hill, NC

**Purpose:** There is growing recognition that environmental factors may play a significant role in determining the health of musculoskeletal tissues, including cartilage. Many of the cellular mechanisms that contribute to cartilage catabolism such as oxidative stress, altered growth factor signaling, matrix mineralization and activation of metalloproteinases can be initiated by heavy metal toxicants. The aim of this study was to determine if heavy metal toxicants activate chondrocyte mitogen-activated protein kinases (MAPKs) that can then lead to altered cartilage homeostasis.

**Methods:** Chondrocytes were enzymatically isolated from commercially obtained 4-6 month-old porcine knee joints (approved by the UNC IACUC) by sequential digestion in pronase and collagenase, and grown in high-density monolayer culture (105 cells/cm²). Cultures were serum-starved overnight and subsequently treated with organic methylmercury (MeHg), inorganic mercuric chloride (HgCl₂), or lead acetate (PbAc), for up to 60 minutes at varying concentrations prior to cell extraction with ice-cold lysis buffer. Additional cultures were treated with IL-1β. Activation of MAPKs was determined by western blotting using phosphorylation-specific polyclonal antibodies.

**Results:** MeHg and caused transient activation of JNK at concentrations as low as 1μM, though the degree of activation by MeHg was lower than by IL-1β. Maximal activation occurred at 10 minutes, returning to baseline levels within 30 minutes. p38 MAPK was activated by IL-1β, but not MeHg or HgCl₂, within this time frame. HgCl₂ and PbAc treatments also caused transient activation of JNK, with maximal activation at 10 minutes.

**Conclusions:** Environmental toxicants are recognized as contributing factors in many chronic human diseases. This study demonstrates that environmental heavy metals activate JNK in articular chondrocytes and suggests that metals may influence the rate or severity of osteoarthritis through modulation of intracellular signaling pathways.

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**P203**

**INHIBITION OF IL-1β-INDUCED HUMAN CARTILAGE DEGRADATION BY POMEGRANATE**

M. Shukla¹, K. Gupta¹, K.A. Khan², C.J. Malemud³, T.M. Haqqi¹

¹Case Western Reserve University, Cleveland, OH, ²Alligarh Muslim University, Aligarh, India

**Purpose:** Pomegranate fruit (Punica granatum L) is revered through the ages for its medicinal properties. Pomegranate fruit (PF) or its extract (PFE) is widely used in Asian and Mediterranean cultures for the treatment of inflammation, for pain in arthritides and other diseases. Osteoarthritis (OA) is the most common musculoskeletal disease among the aging population. Pro-inflammatory cytokine IL-1β plays a dominant role in OA pathogenesis which is characterized by degeneration of articular cartilage of the joints in hands, knees, spine and hips. We have evaluated the effects of a standardized PFE preparation on human OA cartilage and OA chondrocytes treated with IL-1β with a view to determining whether consumption of PF or PFE can be useful in preventing joint cartilage degradation in OA.

**Methods:** Human articular or synovial fluid (SF) obtained at the time of hip arthroplasty. Cartilage explants were prepared by standard methods and chondrocytes were isolated by enzymatic digestion of chondrocytes and obtained in high-density monolayer conditions (105 cells/cm²). Cultures were serum starved overnight and subsequently treated with 1μM, 10μM, or 100μM of IL-1β, or with IL-1β and PFE (10 - 200 μg/ml) using previously described methods. The effect of PFE on IL-1β-induced apoptosis in cartilage was determined by viability assays and Western blotting. Substrate-embedded enzymography and/or collagen degradation assays were used to characterize the MMP activity in conditioned media and SF. Western immunoblotting and immunoprecipitation was used to confirm the presence and/or interaction of specific proteins in the SF and conditioned media. Quantitative RT-PCR was employed to quantify MMP mRNA expression levels. Human chondrocyte nucleofection with reporter constructs was used to analyze the effect of PFE on IL-1β-induced gene promoter activity. Cartilage explants were stained with Safranin-O/Fast Green. Matrix proteoglycan and collagen degradation was determined in culture supernatant using metachromatic assays. The results were analyzed using the Student-t test where p<0.05 was considered significant.

**Results:** PFE at the concentrations used was found to be non-toxic and remarkably effective in inhibiting IL-1β-induced human chondrocyte apoptosis. PFE also blocked the gelatinolytic activities of recombinant human MMPs as well as MMP activity recovered from SF of OA patients. Quantitative zymography also showed that in IL-1β-stimulated human chondrocytes cultures pretreated with PFE the substrate degrading activity of MMP-9 and MMP-13 was significantly lower (p <0.05). Western immunoblotting showed that IL-1β-induced protein expression of MMP-9 and MMP-13 was inhibited when human chondrocytes were pre-treated with PFE. Quantitative RT-PCR showed that PFE blocked MMP-9 and MMP-13 mRNA expression. Furthermore, PFE inhibited IL-1β-induced MMP-9 and -13 gene promoter activity (p <0.005) in transfected human chondrocytes indicating that PFE was acting at the level of transcription. Histochemical and biochemical analysis of human cartilage explants showed that the proteoglycan and Type-II collagen content loss was significantly less (p <0.05) in cartilage explants when PFE was present during IL-1β stimulation or when activated recombinant MMPs were exogenously added to explant cultures pretreated with PFE. Chondrocytes cultured in the presence of PFE also showed increased proteoglycan and collagen synthesis. PFE also blocked the IL-1β-induced loss and inhibition of matrix synthesis.

**Conclusions:** Our results indicated that PFE blocked the IL-1β-induced human cartilage degradation and MMP-9 and MMP-13...
gene expression and activity in vitro. Oral consumption of PFE or active compounds derived from PFE may be protective against cartilage degradation in OA.

**P204**  
**MINIPERL EXPRESSION IN HUMAN CHONDROCYTES WITH A CO-EXPRESSED GREEN FLUORESCENT PROTEIN**  
C. Li, G.R. Dodge  
Nemours Foundation, Wilmington, DE

**Purpose:** Miniperl is an alternative spliced form of perlecan, a proteoglycan that has both structural and functional roles in a variety of tissues including cartilage. Its sequence shares the beginning part with perlecan domain I with exons 4 and 5 spliced out resulting in a frame shift and termination shortly after. The resulting protein shares a portion identical to perlecan and a unique amino acid sequence (AF479675). This study addresses the protein characteristics of miniperl and provides a tool to begin to study its function in both chondrogenesis and in other tissues. Further characterization of this novel chimeric protein will bring new information as to the role(s) that this gene may have in development, normal cell and tissue function, and disease. We used a system that takes the advantages of BM40 signal peptide and GFP for protein monitoring. The tripartite system contained coding sequences for BM40 signal peptide, miniperl, and IRES-EGFP in two reading frames under the control of CMV promoter. Miniperl has been characterized by Western blot and immunoprecipitation with perlecan and miniperl antibodies. Unlike perlecan, in labeling studies performed thus far using osteoarthritic chondrocytes, miniperl does not have an associated glycosaminoglycan chain.

**Methods:** Several steps were performed to include the desired components and prepare the miniperl sequence for insertion into the dual expression vector (with GFP). The miniperl transcript was first inserted into vector pcDNA6/V5-His, then subcloned into the 3’-end of the BM40 signal peptide gene sequence in another vector pCEP-Pu/BMs. The BM40-miniperl-V5-His tag gene sequences were then cloned into pFM2.2 vector. The vector is designated as pFM-BM40-miniperl-V5-His. It was grown in E. coli GC5 cells, prepared with a Qiagen endo-free DNA prep kit. Human chondrocytes were isolated from osteoarthritic patients’ cartilage obtained from NDRI using IRB-approved protocols. Chondrocytes were isolated by routine procedures using collagenase followed by culture in DMEM and grown in 5% CO$_2$ at 37°C. Chondrocytes were cultured in suspension to maintain their cartilage phenotype and transferred for 24 hr to adherent conditions for transfection using the nucleofector kit (Amaxa Inc.). Metabolic labeling and immunoprecipitation were performed with $^{35}$S-labeled sodium sulfate and cysteine-methionine. Rabbit polyclonal anti-miniperl antibodies were prepared to the unique protein sequence.

**Results:** The mammalian expression vector pFM-BM40-miniperl-V5-His was successfully constructed and the correct sequence confirmed by DNA sequencing. The unique properties of this vector allow separate expression of the miniperl and GFP permitting expression monitoring and cell analysis and sorting. Human chondrocytes were transfected with cells in suspension after a 24-hour period of adherence using the Amaxa nucleofector kit. Cell culture medium were harvested and analyzed. Protein of 25-35 kDa is identified on Western blots using perlecan, anti-perlecan domain I-specific, and anti-miniperl antibodies. Immunoprecipitation with perlecan and miniperl specific antibodies indicates the high level expression of miniperl and met/cys labeled bands do not appear labeled with $^{35}$S-NaSO$_4$ indicating the lack of GAG.

**Conclusions:** In this study we generated an efficient construct for the expression of miniperl in human chondrocytes. The tripartite system of BM40 signal peptide, miniperl, and IRES-EGFP in two reading frames under the control of CMV promoter proved the high-level expression and easy detection. Unlike perlecan, labeled miniperl does not have an associated glycosaminoglycan chain in osteoarthritic chondrocytes and additional studies will demonstrate if this is consistent in normal chondrocytes and in other tissues.

**P205**  
**SYNTHESIS AND RELEASE OF CARTILAGE MATRIX PROTEOGLYCANS ARE DIFFERENTLY REGULATED BY NITRIC OXIDE AND PROSTAGLANDIN-E$_2$**  
S.C. Mastbergen, J.W. Bijlsma, F.P. Latber  
University Medical Center Utrecht, Utrecht, The Netherlands

**Purpose:** Recent studies showed a beneficial effect of COX-2 inhibition on proteoglycan turnover of both IL-1β/TNFα induced cartilage damage as well as of osteoarthritic cartilage. Although proteoglycan release and content normalized, proteoglycan synthesis was only partially influenced. Because prostaglandin-E$_2$ (PGE$_2$) is the main product formed by COX-2, this suggests prostaglandin-E$_2$ to play an important role in disturbance a cartilage proteoglycan release but not synthesis, which was subject of the present study.

**Methods:** Human healthy cartilage, alone or in the presence of IL-1β/TNFα (200×800 pg/ml, resp.), was cultured for 7 days with or without prostaglandin-E$_2$ (3 up to 500 pg/ml) or the selective COX-2 inhibitor (celecoxib 10 μM). Changes in cartilage matrix proteoglycan turnover, prostaglandin-E$_2$ and NO were determined

**Results:** Proteoglycan synthesis and release of the cartilage tissue were not affected by prostaglandin-E$_2$ alone. Addition of IL-1β/TNFα to the healthy cartilage resulted in inhibition of proteoglycan synthesis and increased proteoglycan release (p<0.05). When in combination with IL-1β/TNFα, prostaglandin- E$_2$ (200 pg/ml) was added proteoglycan release further increased (p<0.05), but, proteoglycan synthesis was not further influenced. Addition of a selective COX-2 inhibitor could reverse the adverse characteristics of the combination of IL-1β/TNFα treated normal cartilage. The effect was very clear for release, up to almost complete normalization, whereas for synthesis the effect was marginal. In these experiments, celecoxib was also able to normalize the enhanced proteoglycan-E$_2$-levels, whereas enhanced NO-levels remained elevated in the presence of celecoxib. Prostaglandin-E$_2$ levels correlated significantly with proteoglycan release (corr. coefficient: 0.499; p<0.000), whereas NO levels correlated significantly with proteoglycan synthesis (corr. coefficient: -0.626; p<0.000).

**Conclusions:** The present results suggest involvement of prostaglandin-E$_2$ in enhanced cartilage proteoglycan release, although healthy cartilage has to be sensitized by IL-1β/TNFα. IL-1β/TNFα induced NO seems to be more involved in inhibition of proteoglycan synthesis, independent of prostaglandin-E2 and with that insensitive to regulation by (selective) COX-2 inhibitors.