relates to aging-associated changes in cartilage mechanical properties remains unclear.

Methods: Bovine cartilage 8 mm discs, obtained from the weight-bearing area of the femoral condyles were used. Samples were incubated with 0, 2.5, and 7.5 mM threose for a period of 4 days to artificially enhance the AGE levels. On day 4 the center 4 mm of the discs were subjected to physiologic loading under semiconfined conditions (2 MPa, 1800 cycles, 1 Hz) by use of a custom built axial loading device. Subsequently the samples were cultured for an additional 3 days without any additions. Basal mechanical properties (elastic modulus and creep) were determined on day 4 and 7 of the culture. Additional outcome measures included proteoglycan content, proteoglycan release (DMBB assay), AGE levels (pentosidine measurement) and histology (safranin-O staining).

Results: As expected, threose treatment enhanced pentosidine levels in cartilage discs. Physiologic loading did not result in histologically obvious damage to the extracellular matrix. However, there was a loading-dependent decline in proteoglycan content (~17% relative to unloaded controls). This effect was partially blocked by threose treatment, which resulted in a dose-dependent increase in proteoglycan content (+73% relative to untreated controls). At the same time post-loading proteoglycan release decreased with threose treatment (~55%), which was initially increased upon loading (+27%). Consistent with AGE-related stiffening of the extracellular matrix, elastic modulus increased and creep decreased with threose treatment.

Conclusions: These data demonstrate that AGES partially block proteoglycan release associated with mechanical loading of cartilage. Our findings contrast with the general thought that increased stiffness and proteoglycan release decreased with threose treatment (−55%), which was initially increased upon loading (+27%). Consistent with AGE-related stiffening of the extracellular matrix, elastic modulus increased and creep decreased with threose treatment.

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457 CYCLIC TENSILE STRAIN EFFECTS ON SMA3 PHOSPHORYLATION IN HUMAN CHONDROCYTES AND MESENCHYMAL STEM CELLS

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Purpose: Transforming growth factor-β (TGF-β) is involved in chondrocyte proliferation, differentiation and extracellular matrix production. Disregulation of TGF-β1 action has been implicated in cartilage diseases such as osteoarthritis. Moreover, TGF-β1 is a key factor for mesenchymal stem cell (MSC) differentiation toward chondrocytes. TGF-β1 signaling is transduced through a pair of transmembrane serine/threonine kinases leading to phosphorylation and activation of Smad2 and Smad3, which form complexes with Smad4 that accumulate in the nucleus and regulate transcription of target genes.

Mechanical loading has also been shown to be important in chondrocyte extracellular matrix synthesis and MSC differentiation, but mechanisms by which mechanotransduction occurs remain largely elusive. The aim of this work was to analyze the effects of cyclic tensile strain on Smad3 phosphorylation in human chondrocytes and MSC.

Methods: Human articular chondrocytes were obtained after enzymatic digestion of cartilage obtained from osteoarthritic patients undergoing knee replacement. Mesenchymal stem cells were isolated from bone marrow samples obtained from osteoarthritic patients undergoing total hip replacement surgery. Cells were expanded in vitro and then passaged 2 chondrocytes or passed 4 mesenchymal stem cells were seeded on an elastic silicone membrane coated with collagen I. At 80% confluence, cells were pretreated with serum-free medium for 24 h for synchronization. After that, DMEM or chondrogenic medium was added to chondrocytes or MSC respectively; and cyclic tensile strain (CTS, 0.5 Hz, 5% equibiaxial strain) was applied for 10, 30, 60 and 180 minutes using a Flexcell Tension Plus system (FX-4000T, Flexcell International). As a control, cells cultured on the same type of plates, without stretch, were also observed. Using specific antibodies, Smad3 phosphorylation (pSmad3) was quantified on stretched and control cells by western blotting and immunofluorescence analysis was carried out to examine the translocation of transcription factors and genes.

Results: Constitutive activation of ERK 1/2 (p42/44) was not observed in MSCs. Exposure of cells to CTS resulted in a rapid phosphorylation of ERK 1/2 at Thr202 and Tyr204 in the absence and presence of IL-1b that precedes phosphorylation by IL-1b. (B) Densitometric analysis of ERK 1/2 phosphorylation shown in (A). (C) Immunofluorescence analysis of phosphor ERK 1/2 in ACs showing its nuclear translocation. Panel b is negative control for staining, without primary antibody.

Results: In all unloading and loading conditions, Smad2/3 protein complex was detected in the cells (chondrocytes or MSC), with no significant differences in the level of staining or the numbers of cells stained. In unloading conditions, Smad 2/3 was observed in the cytoplasm of cells and few pSmad3 was detected. The application of CTS increases Smad3 phosphorylation in chondrocytes and mesenchymal stem cells at all times tested. However, the increase is maximal at 1 hour of stimulation and decrease after 3 hours of stimulation. In parallel to the increase of Smad3 phosphorylation, Smad2/3 translocation to the nucleus was observed in cells submitted to mechanical stimulation. The effect of CTS seems to be more important in MSC than in chondrocytes.

Conclusions: Our results show that cyclic tensile strain affects smad3 phosphorylation on chondrocytes and mesenchymal stem cells and that the effect is time dependent. CTS could stimulate chondrocyte extracellular matrix synthesis and MSC differentiation by the activation of endogenous TGF-β signaling pathway.

458 RAS ACTIVATION IS REQUIRED FOR ERK MAP KINASES MEDIATED VEGF EXPRESSION BY BIOCHEMICAL SIGNALS IN ARTICULAR CHONDROCYTES

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Purpose: The highly conserved ERK/MAPK kinase pathway is important in cellular responses including cell proliferation, cell differentiation, and cell survival. We hypothesized that anabolic actions (expression of VEGF, HIF-1α, c-Myc) of low/physiological magnitudes mechanical signals are mediated via ERK signaling cascade in chondrocytes. Here we show that biomechanical signals upregulate RAS GTPase activity to initiate ERK signaling cascade and activation of its down stream target transcription factors and genes.

Methods: Articular chondrocytes (ACs) from the knees of 12-14-weeks-old Sprague-Dawley rats were cultured in Ham/F12, 10% FBS, pen/strep and 2 mM L-glutamine, and used in first three passages. Cells (6×10⁴/well) were grown on Bioflex 2 plates for 5 days and subjected to equibiaxial tensile strain (CTS; 3% tension at 0.25 Hz) using Flexcell System (Flexcell Int, NC). Four different treatment regimens were: (i) untreated controls, (ii) cells treated with IL-1β, (iii) cells treated with CTS, and (iv) cells treated with DCF and IL-1β. The regulation of ERK signaling cascade was analyzed by Western blot analysis with phospho-specific antibodies. The confirmation of MAPK activation was carried out by inhibitors of salient molecules in the ERK signaling cascade. Expression of mRNA in response to ERK activation was assessed by real time PCR using Taqman or SYBR green primers and probes. Immunofluorescence analysis was carried out to examine the translocation of transcription factors and MAP kinases in the cells. One-Way ANOVA and the post hoc multiple comparison Dunnett’s test were applied to determine the significance.
CTS-induced RAS activation resulted in c-Raf phosphorylation. However, c-Raf activation was not mediated via PAK phosphorylation and was not inhibited by LY294002. These results suggested that RAS activated c-Raf directly without PI-3-kinase mediated PAK-1 activation. CTS induced c-Raf activation was followed by MEK1/2/Ser217/221 phosphorylation, which in turn phosphorylated ERK 1/2. Interestingly, II-1-induced ERK activation was mediated by RAS GTPase activity, however, its activation cascade was distinct from CTS induced ERK1/2 activation. Examination of downstream events revealed that in parallel to ERK activation, CTS induced an increase in c-Myc phosphorylation and upregulation of c-myc, VEGF, ERK 1/2, aggrecan, gene transcription. Furthermore, inhibition of MEK1 by PD98059 inhibited CTS-induced upregulation of c-myc and VEGF mRNA expression.

**Conclusions:** The results demonstrate that the anabolic actions of mechanical signals, at least in part, are mediated via ERK signaling cascade. Furthermore, CTS upregulated RAS GTPase activity to initiate ERK signaling cascade that results in the induction of genes such as VEGF and c-myc that are associated with chondrocyte growth and proliferation. Acknowledgement. NIHAT00646, AR04878, NIDCRDE15399.

**Modulation of in vitro wear of articulating cartilage: Effect of synovial fluid and cartilage degeneration**

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**Purpose:** One hallmark of osteoarthritis is erosion of the articular cartilage surface. In adult human knee articular cartilage, surface roughening is associated with the weakening of cartilage both in ageing and in age-matched samples. In addition, synovial fluid (SF) exhibits concentration-dependent lubrication qualities for articulating cartilage. Previously, studies of ex vivo joint wear have involved complex joint geometries and non-physiological loading, and in vitro tissue wear has been limited to cartilage apposition against a material such as steel. The hypothesis of this study was that wear of articulating cartilage, under physiologically-motivated loading amplitudes and patterns, increases with the number of loading cycles, decreasing concentration of SF lubricant, and surface fibrillation.

**Methods:** Experiments were performed to assess the effects of number of loading cycles, decreasing concentration of SF lubricant, and cartilage surface fibrillation on the wear of articulating cartilage. For each experiment, the loading protocol was a 1 MPa compressive stress and ±15 mm sliding at 1800 cycles/hr for 4 or 8 hr with n=3-4/group. To assess the direct mechanical effects on wear, test lubricants were spiked with protease inhibitors to inhibit enzymatic activity (anabolic and catabolic) without affecting boundary lubrication. The wear-induced decrease in cartilage thickness and loss of glycosaminoglycan (GAG) and collagen (COL) into the lubricant was determined, and results analyzed statistically with ANOVA. (1) The effect of loading cycles was assessed with bovine samples tested in bovine SF or PBS as lubricant. (2) The effect of SF concentration was determined with bovine samples tested in SF at 100%, 30%, 10%, or 0% concentration. (3) The effect of SF concentration was determined with bovine samples tested in bovine SF or PBS as lubricant.

**Results:** Cartilage thickness decreased with loading cycles, more erosion for samples lubricated with PBS (+260%) than with SF. The rate of GAG and COL loss into PBS occurred in a linear fashion, 0.0027%/cycle (R2=0.98) and 0.00042%/cycle (R2=0.99), respectively. SF lubricant decreased the loss of GAG (~152%) and COL (~377%) compared to PBS lubricant.

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2. Wear increased with decreasing concentrations of SF. Cartilage thickness decrease was 15% with 100% or 30% SF, and increased to 53% (+236% increase above 100% SF) with 0% SF and to 56% (+256%) with 0% SF. Similarly, GAG loss and COL loss, relative to that when lubricated with 100% SF, was higher when lubricated with 30% SF (+64% and +100%), 10% SF (+227% and +233%), and 0% SF (+245% and +250%).

3. Cartilage wear was also dependent on the tissue integrity. The thickness decrease, GAG loss, and COL loss were greater in DGN than NL samples (+62%, +284%, and +190%), and when lubricated by PBS rather than SF (+206%, +165%, and +91%).

**Conclusions:** These studies indicate that altered lubrication and cartilage fibrillation, both, are important factors in the mechanical wear of articulating cartilage. The mechanisms by which SF is wear-protective may involve boundary lubrication to lower wear directly or, indirectly, by lowering friction-induced tissue shear. The increased wear of cartilage with fibrillation may reflect increased shear strain of the collagen network in the superficial region. Enhancement of SF lubrication may be a biomechanical treatment strategy for patients with early or established cartilage degeneration.

**Meniscus, Muscle, Tendon & Ligament Biology**

**Survivorship of collagen meniscus implants (CMI) compared to meniscectomy only: A 5-year analysis in chronic knee patients**

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**Purpose:** Meniscus loss leads to decreased clinical function and activity levels and increases the rate of knee degeneration, thus leading to additional surgeries or even knee replacement, especially in chronic patients. Chronic patients are more focused on preserving their knees and avoiding additional surgeries. The purpose of this study was to determine if replacement of lost or irreparable meniscus tissue with the Collagen Meniscus Implant (CMI) decreased the need for additional surgeries in multiply operated chronic knee patients compared to meniscectomy only. We hypothesized that patients who gained meniscus tissue with the CMI would require fewer surgeries than meniscectomy only controls through five years.

**Methods:** In this prospective randomized multicenter clinical trial (Level of Evidence I), patients 18 to 60 years old who had undergone one to three prior partial medial meniscectomies and currently had clinical symptoms of meniscus pathology were randomized either to receive the CMI or have an additional partial meniscectomy (control). Eighty-five CMI were implanted, but one was removed at 3 weeks after an incision wound infection and two patients died. The remaining 82 CMI patients were compared to 66 controls over 5 years to determine survivorship.

**Survivorship was defined as not having an additional unplanned surgery outside the experimental protocol on the study knee.**

**Results:** Follow-up rate at 5 years was 96%. Eight CMI patients (9.5%) and 15 control patients (22.7%) required reoperation through 5 years. Survivorship at one year was 90% for control and 95% for CMI patients, 86% for control and 95% for CMI patients at 2 years, 83% and 92% at 3 years, 79% for control patients and 91% for CMI patients at 4 years, and 74% for control patients and 89% for CMI patients at 5 years. CMI patients had a significantly higher survivorship compared to controls (p = 0.04). The risk (odds) of reoperation was 2.7 times greater for controls compared to CMI patients at 5 years (95% CI = 1.2 to 6.7). Furthermore, the majority of control patient reoperations occurred prior to 24 months, but only four CMI reoperations occurred during the first 24 months (Figure).

**Conclusions:** This study confirms that chronic patients who received the CMI required fewer additional surgeries in their multiply operated

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