Conclusions: Alkaline phosphatase, BMP-2, and type X collagen production are all characteristic of hypertrophic chondrocytes and may contribute to excess mineralization in OA joints. Taken together, our data suggest that high glucose conditions favor a change toward a hypertrophic phenotype. Ample evidence supports a similar change in human that is characteristic of OA chondrocytes. Our findings are in agreement with the recent clinical studies demonstrating accelerated OA progression in patients with diabetes. The limitations of this study are 1) the hyperglycemic mice were slightly heavier than controls which may add mechanical effects (adipose levels were not measured); and 2) higher noise-to-signal background was present with the type X collagen antibody. These are balanced by the strengths of this study which include 1) animals were verified hyperglycemic prior to DMM, and 2) phenotypic change was tested in two model systems (in vivo rodent and in vitro large mammal). In summary, this study suggests the hypothesis that accelerated hypertrophy of articular chondrocytes is a result of hyperglycemia and may contribute to the progression of OA as previously seen in patients with diabetes.

418 EFFECTS OF FIBROBLAST GROWTH FACTOR-2 AND ITS RECEPTOR ANTAGONISTS IN OSTEOARTHRITIS

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Purpose: The fibroblast growth factor (FGF) family represents an interesting group of molecules which are involved in the regulation of connective tissue development and metabolism. FGFs signal through receptors FGFRI-4. FGF-18, which has been shown to signal principally through FGRF3, has promising beneficial effects in articular cartilage, whereas FGF-2 has been shown to signal also through other FGF receptors. The role of FGF-2 in osteoarthritis (OA) remains unknown in many aspects. In the present study we investigated the presence and effects of FGF-2 in OA joints by assessing the associations of FGF-2 with cartilage degrading matrix metalloproteinase (MMP) enzymes and with the synthesis of the major cartilage matrix components aggrecan and collagen II as well as by investigating the effects of FGF receptor antagonists.

Methods: Synovial fluid and cartilage samples were obtained from 97 OA patients undergoing total knee replacement surgery (60 females and 37 males, BMI 30.9 ± 0.6 kg/m², age 69.8 ± 1.0 years; mean ± SEM). FGF-2 concentrations in the synovial fluid and cartilage culture medium were measured by immunoassay. FGF receptor expression profiles of primary human OA chondrocytes were determined by quantitative RT-PCR. The effects of FGF-2 and its receptor antagonists on the expression of MMPs and collagen II were investigated in cultures of primary human OA chondrocytes. The study was approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland and it was carried out in accordance with the Declaration of Helsinki. The patients gave their written informed consent, and their diagnosis was confirmed to fulfill the ACR classification criteria for osteoarthritis.

Results: FGF-2 was present in OA synovial fluid and released into the culture media from cartilage samples obtained from OA patients. Interestingly, FGF-2 concentrations correlated positively with the concentrations of MMP-1 (r = 0.414, p < 0.001) and MMP-13 (r = 0.362, p < 0.001) in the cultures of OA cartilage. In primary human OA chondrocytes FGF receptors 1-4 were expressed, with the expression of FGFRI being the highest. FGF-2 up-regulated the production of MMP-1 and MMP-13, and down-regulated the expression of aggrecan and collagen II, in human OA chondrocyte cultures. More importantly, FGF receptor antagonists AZD4547 and NVP-BGJ398 (10-300mM) down-regulated the production of MMP-1 and MMP-13 and up-regulated the expression of aggrecan and collagen II in a concentration-dependent manner, and not only in the presence but also in the absence of exogenous FGF-2.

Conclusions: The present results suggest that, in contrast to its growth factor like effects in some other conditions, FGF-2 induces catabolic and anti-anabolic effects in OA joints by up-regulating the production of matrix degrading enzymes MMP-1 and MMP-13 and downregulating the de novo synthesis of cartilage matrix components aggrecan and collagen II. Moreover, by counteracting these effects FGF-receptor antagonists showed promising beneficial effects on the balance of catabolic and anabolic mediators within OA cartilage.

419 INTRINSIC CARTILAGE REPAIR BY JOINT DISTRACTION IS TRIGGERED BY A REGENERATIVE TRANSCRIPTIONAL RESPONSE


Purpos e: Joint distraction provides long-term improvement of pain and function, accompanied by intrinsic cartilage repair evaluated indirectly by imaging and biochemical markers in humans with knee osteoarthritis (OA) [Wiegen et al., 2013]. Moreover, joint distraction results in cartilage repair activity in an experimental canine model of OA, which corroborates with the structural observations of cartilage repair by surrogate markers in humans [Wiegen et al., 2014]. Despite these promising results little is known about the exact mechanisms that boost them. This study evaluates for the first time the regenerative transcriptional response during joint distraction in an experimental model of osteoarthritis.

Methods: Knee OA was induced in 8 dogs according to the groove model. Ten weeks after OA induction, 4 dogs received joint distraction (D) and 4 dogs served as disease controls (OA). After 4 weeks of distraction the animals were sacrificed and knee tissues including cartilage samples were obtained by fat pad, synovium, meniscus, bone and cartilage were harvested. qPCR analysis of more than 35 state-of-the-art regenerative gene markers was performed.

Results: The OA group revealed an upregulation of typical OA markers, like matrix metalloproteinases, collagen, and apoptosis markers confirming the OA disease cascade in specifically cartilage, bone and synovial tissue. Joint distraction caused downregulation of the typical OA markers and the maintenance of some important matrix remodeling genes for regeneration, e.g. aggrecanase. Moreover, genes from several pathways were differentially expressed between the D and OA group, including TGF-β1, Wnt, and Notch signaling pathways. Interestingly, a high number of events occurred in bone, highlighting the importance of this tissue in the regenerative outcome of joint distraction on OA cartilage.

Conclusions: Distraction is a good candidate for knee OA treatment resulting in prolonged clinical and structural changes. This study demonstrates distinctively that joint distraction initiates a transcriptional regulation of several important regenerative genes indicating that a reset of joint homeostasis can lead to cartilage repair in OA.

420 THE INFLUENCE OF RE-MOBILIZATION ON DEGENERATED CARTILAGE INDUCED BY JOINT IMMobilIZATION – PATHOLOGICAL PROCESS OF THE CYST FORMATION


Purpose: Understanding of the degeneration process in articular cartilage is necessary to develop strategies to prevent and treat joint disease and cartilage degeneration. Joint immobilization causes various degeneration of articular cartilage such as thinning, softening and reducing proteoglycan content. We reported that degenerated cartilage after 8-week immobilization was aggravated by re-mobilization, which is characterized as cyst formation. The degenerated cartilage was not observed in the contact area between the femoral condyle and the tibial plateau but marginal of the contact area [Nagai et al., 2014 World Congress on Osteoarthritis]. However, it is still unknown when and how the cyst formation occurs. The purpose of this study was to examine when the cyst formation occurs and the pathological process of the degeneration induced by re-mobilization.

Methods: The left knee joint of Wistar rats aged 12-week old was immobilized at 140 ± 5 degrees of knee flexion with K-wire and resin for 8-week. Thereafter the external fixator was removed and bred again for 5-, 6-, 7- and 8-week. Control group applied K-wire in the femur and tibia diaphyses as a sham operation, but the joint motion was not restricted. Forty rats were prepared for histological analysis (experimental group: n = 5/each period, control group: n = 5/each period). The rats were sacrificed at the end of experimental period. The knees were extirpated, fixed with 4% PFA, decalcified in 10% EDTA and
embedded in paraffin. Six-μm sections were obtained at the medial mid-condylar segment of the knee in the sagittal plane. The sections were stained with hematoxylin-eosin and safranin-O (SO). The modified Mankin’s score was applied for histological evaluation of the cartilage degeneration. At two areas (contact area and transitional area) which located between non-contact and contact) in tibia. About the calcified cartilage and the non-calcified cartilage in each assessment area, the expression intensity of type II collagen by immunohistochemical analysis and SO staining intensity were measured.

**Results:** In experimental group, the cyst formation was observed in transitional area only at 8-week, whereas the degeneration was not observed before 8-week and in contact area. This degeneration existed in the non-calcified cartilage, where the increased hypocellularity was observed with extension of re-mobilization period. However, in the calcified cartilage of the same area, the hypocellularity was not observed. At transitional area in experimental group, the SO staining intensity in both non-calcified and calcified cartilage were decreased throughout experimental period in comparison with the control group (P < 0.05). Although there was no significant difference between the time points, the intensity was decreased after 6-week in both non-calcified and calcified cartilage. On the other hand, the type II collagen expression intensity in both non-calcified and calcified cartilage did not show significant difference between the experimental and the control group and between the time points. About contact area in experimental group, the decreased SO staining intensity were observed at both non-calcified and calcified cartilage throughout experimental period in comparison with the control group (P < 0.05), especially, remarkable reduction of SO staining intensity was observed at 8-week. The collagen II expression intensity did not show significant difference between the experimental and the control group and between the time points, similarly to that at transitional area. The modified Mankin’s score at both contact and transitional areas in experimental group was higher than that in the control group throughout experimental periods (P < 0.01). However, there was no significant difference between the time points.

**Conclusions:** Current results showed that the cyst formation would have occurred at non-calcified cartilage between 7 to 8 weeks after remobilization. It might suggest that the cyst formation would occur when degeneration of non-calcified cartilage exceed a certain threshold, however the pathology of cyst formation was not clear in this study.

**Mechanobiology**

**421 THE POSSIBLE ROLE OF VASCULO-MECHANICAL FACTORS IN JOINT PATHO-PHYSIOLOGY**

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**Purpose:** Historically intraosseous pressure (IOP) was found to be raised in early arthritis and osteonecrosis. Steroid use and embolic bone diseases were also associated with a raised IOP. More recent research has suggested that subchondral region may be important in cartilage nutrition. It is probable that high pressures occur in the subchondral region of weight bearing joints. This study was carried out to explore the mechanical and hydrodynamic forces around joints in use. A stepwise approach was used to explore subchondral bone perfusion.

**Methods:** IOP was measured initially in the cancellous bone of the femoral head, femoral condyle and upper tibia of anaesthetised adult New Zealand White rabbits. A needle was inserted into subchondral bone and connected by a saline filled line to pressure transducers and a chart recorder. Clamps could be placed on the proximal femoral artery and vein. A load of one body weight was applied longitudinally through the limb as required. Similar upper tibial IOP measurements were made in conscious walking volunteers.

**Results:**

1. Basal intraosseous pressure varied widely from 12-60 mmHg with a cardiac pulse volume (PV) of 3-10 mmHg. In 43 separate studies there was a close correlation between the IOP and its associated pulse volume, r = 0.801, p<0.001. There was also an underlying respiratory wave (RW).
2. Drugs affected IOP, closely reflecting the systemic circulation pressure changes.
3. Occlusion of the proximal femoral artery causes loss of pressure (IOPa) and pulse volume to virtually nil.
4. Occlusion of the proximal vein causes a rise in pressure (IOPv) with preservation of PV and RW.
5. One body weight load raises IOP with preservation of PV and RW.
6. During arterial occlusion loading caused very little rise in IOPv.
7. During venous occlusion loading caused an augmented rise in IOP with preservation of the PV.
8. Perfusion at the needle tip is best understood as a function of IOPv minus IOPa.
9. Simultaneous recordings from the femoral head, condyle and upper tibia during vascular occlusion and loading show the same changes at all sites.
10. Triple recording from the femoral head, condyle and upper tibia during injection of saline shows pressure is transmitted through each whole bone but not across the joint.
11. In man upper tibial pressure during standing, slow walking and fast walking shows large IOP changes of up to 1000mmHg.

**Conclusions:** IOP is mainly a reflection of arterial supply pressure and not venous back pressure. When IOP is studied combined with alternate proximal arterial and venous occlusion, actual cancellous bone perfusion at the needle tip can be studied. A single IOP measurement in isolation is meaningless. Compartment syndromes could probably be studied in the same way IOPv – IOPa.

IOP is reduced by proximal arterial occlusion and increased by proximal venous occlusion and physical loading.

There is an element of compartmentalisation in each bone. Bones are hydrodynamically separated by joints. High pressure fluctuations arise under subchondral bone during weight bearing activity. There may be protective anatomical modifications of the subchondral bone microcirculation. Arthritis may be a partly ‘vasculo-mechanical’ disease. Subchondral bone appears to act as a compressible perfused sponge.

**422 MECHANOBIOLOGICAL RESPONSE OF ARTICULAR CARTILAGE SUBJECTED TO SIMULTANEOUS COMPRESSION AND SLIDING**

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**Purpose:** The biological and mechanical response of articular cartilage to stationary compressive and shear forces has been extensively studied. In physiological conditions however, joint locomotion involves continuously changing contact areas over the tissue surface. Such migrating contacts play an important role for the durability and wear-resistance of the tissue. By allowing the contact area to migrate over a cartilage surface, stress and strain become dynamic parameters depending on the sliding load applied and the contact location. In this study, we investigate the mechanobiological response of articular cartilage over the application of different axial loads at a physiological sliding velocity. We hypothesized that increasing axial load would lead to an up-regulation of genes associated with extracellular matrix remodeling.

**Methods:** Fresh mature bovine femoral condyles were removed from the knee and mounted into our custom designed cartilage-sliding machine. An axial load was applied and displacements in x-(anterior-posterior, AP) and y-(proximal-distal, PD) directions measured. The cartilage surface was mapped by repeatedly lowering a delrin indenter (r = 12.7 mm) onto the surface. A stress-strain curve could then be obtained for each location to determine the surface geometry and mechanical properties. Sliding (± 36 mm amplitude) at 10 mm/s and one of 4 normal forces (12, 24, 36 and 48 N) was applied for 400 cycles (48 minutes). Experimental and unloaded (control) samples were harvested and incubated for 1h before analyzing for gene expression by RT-qPCR. The bone surface was also mapped to determine the thickness of the cartilage. For each specimen harvested, strain, stress and dynamic modulus after 400 cycles were calculated according to Hertzian theory of elastic deformation. A linear regression model was used to correlate strain, stress and dynamic modulus with gene expression values of the following genes: collagen type Ila, aggrecan, fibronectin, sox-9 and MMP-3.

**Results:** All condyles undergo creep deformation, reaching “dynamic equilibrium” after 15–25 cycles of loading (Figure 1).