incubated with the calcium sensitive fluorescent dyes: Fura Red and Fluo-4 and subsequently attached to a glass coverslip and immersed in DMEM in a heated (37°C) perfusion chamber. Confocal images were collected every 3.5 seconds for 12 minutes.

Following the first 9 scans, the DMEM was removed and replaced with 0.1, 1, 10 or 55 ng/mL IL-1α or the corresponding vehicular control (PBS). A [Ca2+]i response was defined as an increase in the fluorescence ratio Fluo 4/Fura Red > 3 sd above background measured during the first nine scans. The percentage of cells responding with single or multiple fluxes was determined and statistically analyzed (2, p < 0.05).

Results: Thirty percent of chondrocytes responded with [Ca2+]i, when exposed to 1, 10 or 55 ng/mL IL-1α. This response was consistently and significantly greater than the corresponding vehicular controls. In contrast, there was no significant effect at 0.1 ng/mL IL-1α (Fig. 2).

Conclusions: Our data suggests that 1 ng/mL is the threshold concentration of IL-1α which exerts a significant effect on chondrocytes in intact murine femora. Such a threshold response may imply the activation of the chondrocyte immunoglobulin IL-1 receptor. Thirty percent of chondrocytes responded with [Ca2+]i, fluxes in a dose independent manner, perhaps indicating a saturation of IL-1 receptors at 1 ng/mL IL-1α. This is in contrast to previous studies that have imaged chondrocytes in explants or culture and reported a larger percentage of cells responding in a dose dependent manner (24% at 0.1 ng/mL, 90% at 10 ng/mL in explants). Notwithstanding species (porcine/murine) and diffusion contrasts between these studies, we hypothesise that there is a saturation of the IL-1 receptors at 1 ng/mL IL-1α in intact murine cartilage and that the integrity of the extracellular matrix interactions with both the subchondral bone and chondrocyte membrane influence both the number of chondrocytes responding and the dose dependent characteristics of this response. In conclusion, these data provide further evidence that the increased concentration of IL-1α present in osteoarthritic joints has the ability to influence the biological activity of chondrocytes in vivo.

265
GLUCOSAMINE SULFATE REDUCES THE PROSTAGLANDIN E2 PRODUCTION IN OSTEOARTHRITIC CARTILAGE THROUGH THE INHIBITION OF mPGES-1
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Purpose: Among the prostaglandins of the E series, PGE2 is one of the major inflammatory/catabolic mediators involved in cartilage degradation associated with osteoarthritis (OA). Moreover, several of the effects of the catabolic cytokine, IL-1β, are mediated through the stimulation of PGE2 production. As glucosamine sulfate (GS) has been inferred to have a potential anti-inflammatory effect on OA symptoms, we explored its effect on PGE2 in human OA chondrocytes and at which level in the PGE2 pathway its effects takes place. This pathway includes the cyclooxygenases (COX)-1 and -2, the terminal enzyme responsible for PGE2 synthesis, mPGES-1, as well as its co-factor glutathione and its transcriptional signalling pathway, the early growth response factor (Egr)-1. In addition, as PPAR-γ activation inhibits IL-1β induced mPGES-1, we also examined the effect of GS on this factor.

Methods: The effect of GS treatment (0.2, 1, and 2 mM) on human OA chondrocytes (n=5–8) was investigated in the absence or presence of IL-1α (100 pg/ml). The expression levels (real time PCR) and protein production/activity of PGE2, COX-1, COX-2, mPGES-1, glutathione, Erg-1, and PPAR-γ, using specific primers (expression), antibodies or assays (protein), were determined.

Results: Data showed that GS treatment at 1 and 2 mM significantly inhibited (p≤0.03) the basal endogenous and IL-1β-induced PGE2 production. GS in both the absence and presence of IL-1β did not significantly modulate COX-1 protein production but, interestingly, GS at 1 and 2 mM demonstrated a decrease in COX-2 activity in that it reduced the molecular mass of COX-2 synthesis from 72–74 kDa to 66–70 kDa. Under IL-1β stimulation, GS significantly inhibited the mPGES-1 mRNA expression and synthesis at 1 and 2 mM (p≤0.02) as well as the activity of glutathione (p≤0.03) and the Erg-1 (p≤0.05) at 2 mM. Finally, data showed that in the absence and presence of IL-1β, PPAR-γ was significantly induced by GS at 2 mM (p≤0.02).

Conclusions: Our data further documents the potential mode of action of GS at reducing the catabolism of human OA cartilage. GS inhibits PGE2 synthesis via a reduction in the activity of COX-2 and the production and activity of mPGES-1. These findings may help explain the mechanisms by which this drug exerts its positive effect on OA pathophysiology. This study was supported in part by a grant from Rottapharm.

267
ROLE OF POLYAMINES IN HYPERTROPHY AND TERMINAL DIFFERENTIATION OF OSTEOARTHRITIC CHONDROCYTES
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Purpose: Polyamines are naturally occurring, positively charged polycations able to control several cellular processes in different cell types, by interacting with negatively charged compounds and structures within the living cell. Several pieces of evidence from the literature point at a role of polyamines in promoting chondrocyte differentiation. Hence, we investigated the effects of exogenously added spermine or spermidine in chondrocyte maturation recapitulated in 3D micromass cultures, to tease out the effects on gene and protein expression of key chondrogenesis regulatory transcription factors, markers and effectors, as well as their posttranscriptional regulation.

Methods: Micromasses were seeded in control medium or in the presence of either 5 μM spermine or spermidine. We evaluated the effects on molecular markers of chondrocyte differentiation at the level of gene (real time PCR) and protein (western blot and immunohistochemistry) expression as well as the effects on extracellular matrix deposition and remodeling, and caspase activation and mineralization. The subcellular localization of RUNX-2 in stimulated samples was investigated with confocal microscopy.

Results: Both spermine and spermidine were able to increase the rate and the extent of chondrogenesis, with enhanced collagen 2

Fig. 1. An overlay of the Fura Red and Fluo-4 fluorescent calcium images of chondrocytes on a condyle of an intact murine femora. Scale bar = 50 μm; original magnification 40× and 512×512 pixel resolution.

Fig. 2. Graph of the percentage of cells responding as a function of IL-1α concentration or vehicular control (PBS). Single or multiple [Ca2+]i responses are indicated. a = significantly different from the corresponding vehicular control (p < 0.05). Data from >200 chondrocytes from 3 independent femora were analyzed in each experimental group.

Poster Presentations / Osteoarthritis and Cartilage 19S1 (2011) S53–S236
deposition and remodeling with downstream generation of collagen 2 bioactive peptides. These were able to promote nuclear localization of RUNX-2, the pivotal transcription factor in chondrocyte hypertrophy and osteoblast generation. Indeed, samples stimulated with polyamines showed an enhanced mineralization, along with increased caspase activity, indicating increased chondrocyte terminal differentiation.

**Conclusions:** Polyamine pathway can represent a potential target to control and correct chondrocyte inappropriate maturation in osteoarthritis.

**Acknowledgements:** supported by CARISBO foundation

### 268 EXPRESSION OF P53R2 IN CHONDROCYTES IS REGULATED BY MECHANICAL STRESS
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**Purpose:** p53 tumor suppressor protein is activated in response to DNA damage. Ser46 residue of p53 is phosphorylated and p53AIP1 (apoptosis inducing protein) induced apoptosis in case of severe DNA damage. While the damage is not so severe, Ser15 residue of p53 is phosphorylated and p53R2 repaired DNA damage. We previously showed that excessive mechanical stress induced chondrocytes apoptosis via p53 and p53AIP1 pathway. In this study, we evaluated p53R2 expression and function of chondrocytes in response to mechanical stress.

**Methods:** OA cartilage samples were obtained from total knee replacement surgery, and normal cartilage samples were from femoral neck fracture. The expression of p53R2 was analyzed by immunohistochemistry. Chondrocytes were isolated from OA and normal cartilage. The expression of p53R2 in chondrocytes was detected by western blotting and real-time PCR. OA chondrocytes were introduced 2, 5 and 10% tensile strain for 12 hours by using FX-2000. After the strain, expressions of p53R2, type 2 collagen and aggrecan were detected by real-time PCR. The phosphorylation of Ser15 and Ser46 residue of p53 was analyzed by western blotting. p53R2 siRNA was transfected to OA chondrocytes, and expressions of type2 collagen and aggrecan were detected after tensile strain.

**Results:** p53R2 was highly expressed in OA cartilage in comparison with normal cartilage by immunohistochemistry. Western blotting and real-time PCR showed p53R2 expression in chondrocytes was higher in OA chondrocytes than in normal chondrocytes. p53R2 expression in OA chondrocytes was increased after 2 and 5% tensile strain and decreased after 10% tensile strain. P53R2, Type2 collagen and aggrecan expressions were increased after 2 and 5% tensile strain but decreased after 10% strain. Ser15 residue of p53 was phosphorylated after 5% strain, but Ser46 residue was not. After the transfection of p53R2 siRNA, expressions of type2 collagen and aggrecan were down-regulated.

**Conclusions:** In our study, p53R2 in chondrocytes was increased after 2 and 5% tensile strain. Down-regulation of p53R2 reduced type 2 collagen and aggrecan expression via Ser15 of p53 in response to mechanical stress. Up-regulation of p53R2 may increase type 2 collagen and aggrecan expression via Ser15 of p53 in response to mechanical stress. We consider that regulation of p53R2 might be one of strategy for OA treatment. We are doing further investigation to analyze p53R2 function in chondrocytes.

### 269 CHONDROCYTE CLUSTER FORMATION STIMULATES CARTILAGE MATRIX DEPOSITION
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**Purpose:** Cell cluster formation in articular cartilage is a hallmark in Osteoarthritis (OA). These cell clusters have been associated with both cartilage catabolism and anabolism. We addressed this issue by investigating the effect of clustering chondrocytes in highly controlled micro-aggregates on their morphology, stability and chondrogenic potential.

**Methods:** We designed a micro-mold that enables controlled formation of micro-aggregates ranging from 20 to 200 cells in high throughput. Morphology, stability and chondrogenic potential of micro-aggregates was evaluated and compared to single-cells cultured in micro-wells and in 3D after encapsulation in a Dextran-Tyrannine (Dex-TA) hydrogel in vitro and in vivo after subcutaneous implantation in mice.

**Results:** We successfully formed micro-aggregates with highly controlled size, morphology, cell density, stability and viability. Micro-aggregates of 100 cells presented a superior balance in Collagen type I and Collagen type II gene expression over micro-aggregates of 20 and 50 cells. Matrix metalloproteinase (1, 9 and 13) gene expression was decreased in micro-aggregates compared to single cells. Histological analysis of hydrogels cultured in vitro and after implantation in mice demonstrated enhanced matrix deposition in constructs seeded with micro-aggregates, compared to single-cell seeded constructs.

**Conclusions:** Using a highly controlled model for high throughput formation of micro-aggregates, we demonstrated that clustering chondrocytes in micro-aggregates stimulated cartilage matrix formation. Consequently, the use of micro-aggregates, compared to single cells, resulted in greatly improved neo-cartilage formation. Furthermore, our data provided experimental evidence suggesting that cartilage clusters found in osteoarthritic cartilage are part of a regenerative response.

### 270 FEASIBILITY OF CHONDROCYTE CULTURES FROM CADAVER FINGER JOINTS
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**Purpose:** Chondrocyte cultures are instrumental for studying the pathogenesis of osteoarthritis of the hands. We investigated the feasibility of using human cartilage from fingers of dissecting room cadavers donated to the institute of anatomy.

**Methods:** Proximal interphalangeal joints (PIP) were obtained from 17 untreated dissecting room cadavers (mean age 66) and a post mortem time up to 101 hours. The joint surface appeared macroscopically osteoarthritic in four cases. Cartilage and connective tissue was harvested under sterile conditions. As a control we used cartilage derived from patients undergoing total knee joint replacement. Tissues were digested in collagenase B and cultured in Ham's F-12/DMEM (1:1) and 10% FBS over 3 passages. Gene expression of matrix metalloprotease (MMP)-13, inducible nitric oxide synthase (iNOS), Collagen II and X and alkaline phosphatase was evaluated using quantitative real-time PCR and western blot.

**Results:** Chondrocytes from cadavers up to 101 hours post mortem were viable in all cases. Cell yields were comparable to controls with an average of 3*10^6 cells after two cell culture passages. RNA was isolated with an average of 500 ng to 1000 ng. Chondrocytes from PIP exhibited typical histological morphology and expression of Collagen II when compared to fibroblasts. In comparison with articular chondrocytes from patients undergoing knee joint replacement cultured chondrocytes from PIP had significantly lower basal expression of iNOS (48 fold, P < 0.01) and Collagen X (3.7 fold, P < 0.01). In contrast basal gene expression of alkaline phosphatase were found to be elevated 15 fold (P < 0.01) on hand OA chondrocytes. Expression of MMP-13 was not significantly different.

**Conclusions:** Cadaver chondrocyte culture from finger joints is feasible. In this pilot study, chondrocytes from hand osteoarthritis showed differences in the expression of iNOS and collagen genes.

### 271 AGING AND OXIDATIVE STRESS REDUCE THE CHONDROCYTE RESPONSE TO IGF-1 AND OP-1
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**Purpose:** The mechanisms by which aging changes in the joint predispose older adults to develop OA are incompletely understood. Oxidative stress is thought to play a role in age-related conditions. The purpose of this study was to determine the effects of age and oxidative stress on the chondrocyte response to IGF-1 and OP-1, two important anabolic factors in cartilage.

**Methods:** Human articular chondrocytes were isolated from normal talar cartilage obtained from over 40 tissue donors with ages ranging from 19–91yrs. Cells in confluent primary cultures or in alginate beads were made serum-free and stimulated with 50–100 ng/ml IGF-1, OP-1, or