synovial membrane nodules showed blood vessels containing red blood cells, as well as, osteoclasts. Type I and type IIb loose bodies, however, did not show them.
Conclusions: It is well known that loose bodies grow from proliferation of cartilage without blood supply in the joint cavity, and that enchondral ossification is able to develop only under the condition of having a blood supply. As synovial membrane nodules were also classified to the same type as loose bodies and more than half of osteochondral and osseous loose bodies contained blood vessels with red blood cells, the loose bodies were thought to be caught in the synovial membrane and to be modified as the result of a blood supply. Considering the results of this study, various histologic characteristics of loose bodies in osteoarthrosis resulted from modifications including cartilage proliferation in the joint cavity and enchondral ossification in the synovial membrane.

150 INHIBITION OF CELL DEATH PREVENTS CARTILAGE DEGRADATION IN ACUTE TRAUMA MODEL

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Purpose: Post-traumatic arthritis is one of the most common causes of secondary osteoarthritis (OA). The aim of our study was to investigate the effect of anti-apoptotic agents (non-ionic surfactant P188 and inhibitors of caspases 3 and 9) on cartilage degradation after a single impact to human ankle cartilage.
Methods: Human normal tali (cartilage with bone attached) obtained from organ donors were impacted with a nominal stress of 25−30 MPa. Cartilage from both left and right ankles was used; one served as an un-impacted control and a second was impacted with a 4 mm indenter. Full thickness 8 mm cartilage plugs consisted of impacted (4 mm core) and immediately adjacent (4 mm ring) areas were removed and either collected at day 0 or cultured with or without P188 (8 mg/ml), caspase 3 inhibitor (10 uM), or caspase 9 inhibitor (2 uM). The effect of treatments was assessed at days 0, 1, 2, 7 and 14 post injury by live/dead cell assay, apoptosis (Tunel Stain), histology with Safranin O staining, and proteoglycan content released into the media. At each time point histological assessment with a modified Mankin score and the number of viable and apoptotic cells were evaluated in the superficial and middle-deep layers separately.
Results: An impact to articular cartilage caused cell death by necrosis primarily in the superficial layer of the impacted core. By day 14 of culture, the number of dead cells in this area was increased by more than 30%. The impact also caused cell death by apoptosis which was observed in both, impacted core and adjacent ring. Tunel-positive chondrocytes were present in all cartilage layers. During culture, cell death by apoptosis spread out to the areas that did not experience the impact and the number of apoptotic cells was statistically increased by day 14 not only in the core, but also in the ring. All three treatments improved cell survival by necrosis at all time points. However, with regard to apoptosis, P188 appeared to be the most effective among chosen treatments: already at day 2, the number of Tunel-positive chondrocytes was significantly reduced in the superficial layer of both core and ring (13% $\pm$0.58 and 9%$\pm$4.8 respectively) in comparison to the non-treated group (33%$\pm$5.49 and 30%$\pm$0.53 respectively, $p < 0.01$). It also inhibited expansion of apoptosis through the adjacent ring area. Within the first 7 days, P188 was able to maintain apoptosis in the ring at a steady level (about 20% of Tunel-positive cells) vs 60% ($p < 0.05$) detected in the untreated control. Among caspase inhibitors, anti-caspase-3 was more effective in reduction of apoptosis than anti-caspase-9. P188 and caspase-3 inhibitor exhibited a similar effect on cartilage integrity. Mankin score under both treatments remained within a normal range (4$\pm$0.5), while in the untreated core it was 7$\pm$0.5. Similar results were observed for the ring area. The caspase 9 inhibitor did not improve histological appearance of cartilage sections. Only the P188 treatment significantly reduced the amount of proteoglycans released into the media at all time points.
Conclusions: This study documented that P188 and caspase 3 inhibitor were effective in promoting chondrocyte survival and thus protecting cartilage from degeneration in acute trauma model. Combination of these anti-catabolic treatments with anabolic agents may provide novel therapeutic approaches to stimulate proper cartilage repair in order to prevent post-traumatic OA.

151 SMALL HEAT-SHOCK PROTEINS, NOVEL IDENTIFIED MEDIATORS OF MATRIX GENE EXPRESSION, ARE DIFFERENTIALLY EXPRESSED IN OA CHONDROCYTES

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Purpose: αBcrystallin (HSPb5) and HSP27 (HSPb1) belong to the family of small heat-shock proteins. This family of proteins is only poorly described in chondrocytes. We aimed to investigate the expression levels of these proteins in chondrocytes isolated from healthy and osteoarthritis (OA)-affected patients. The functional role of αBcrystallin in chondrocyte metabolism was further explored using RNA-interference.
Methods: Western blot and real-time RT-PCR analysis were performed to determine the expression levels of αBcrystallin and HSP27 in healthy and OA chondrocytes cultured in alginate beads. RNA-interference mediated gene knock-down was used to explore the role of αBcrystallin in chondrocyte biology by transfecting low concentrations of siRNA directed against the αBcrystallin sequence in cultured chondrocytes. To determine the differentiation status dependent expression of small heat-shock proteins, phenotypically stable chondrocytes were seeded in low-density monolayer cultures to allow expansion and partial dedifferentiation. Expression levels of αBcrystallin and HSP27 were monitored at different time points.
Results: Based on previous proteome screening analyses, αBcrystallin and HSP27 were selected as potential interesting proteins in the development of OA. A decreased abundance of these proteins was observed in OA chondrocytes by Western blot. Moreover, real-time RT-PCR confirmed the differential expression between chondrocytes isolated from visually intact and visually damaged zones of OA cartilage from the same patient. The pro-inflammatory cytokines Il-1β and TNF-α, both down regulated αBcrystallin and HSP27 expression. Transfection of low concentrations siRNA in cultured chondrocytes resulted in an efficient knock down of αBcrystallin gene expression. This was accompanied by an altered expression of the chondrocyte specific genes BMP-2, aggrecan and collagen type II. The observed reduced expression of Aggrecan, COL2A1 and BMP-2 may reflect that a decreased expression of αBcrystallin is associated with a more dedifferentiated cellular phenotype. This hypothesis is supported by our observation that αBcrystallin showed an initial decreased expression in monolayer-cultured dedifferentiating chondrocytes.
Conclusions: In conclusion, our study clearly demonstrates the differential expression of small heat-shock proteins on the mRNA as well as on the protein level between healthy and OA-affected articular chondrocytes, suggesting a distorted chaperone response in OA. This differential expression seems to be a consequence of a cytokine driven mechanism. Additionally, our data disclose αBcrystallin as a novel identified regulator of matrix gene expression. The association between reduced αBcrystallin levels and reduced levels of aggrecan, collagen type II and BMP-2, further adds to the evidence that this protein might be involved in phenotypic changes of chondrocytes during the development of OA.

152 *IN VITRO* VERSUS *IN VIVO* EXPOSURE OF CARTILAGE TO BLOOD

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Purpose: Joint bleeds lead to joint destruction. Knowledge about the mechanism of this blood-induced arthropathy has originated from both *in vitro* and *in vivo* studies. Our group has shown that *in vitro* exposure of cartilage to 50% v/v blood for 4 days leads to severe (−98%) and long-lasting (−78% after 16 days) inhibition in cartilage matrix synthesis. Also after an experimentally *in vivo* induced haemorrhage in the dog knee joint, direct harmful effects were observed, including inhibition of the cartilage matrix synthesis (−22%). But while in the *in vitro* experiments this inhibition was long lasting, in the *in vivo* experiments, effects were less outspoken and long-lasting. One of the differences between the *in vitro* and the *in vivo* situation is that in the *in vivo* situation, the cartilage is exposed to blood at the articular surface only, whereas in the *in vitro* explant culture system the cartilage is exposed to 5 additional cutting edges. Whether this difference in exposure of cartilage to blood can explain the difference between the *in vitro* and *in vivo* studies on blood-induced cartilage damage was subject of this study.
Methods: Human articular cartilage explants were exposed to blood either totally, by submerging explants in 50% v/v blood, or to the articular surface alone. For this purpose a specific culture device was developed and validated. The effects of this exposure on the proteoglycan synthesis rate and -release were determined.