the cytoplasm of live cells green, Propidium iodide - stains the nuclei of dead cells red) were used to determine *in situ* chondrocyte viability at 2.5 hours (controls) and 7 days. Percentage cell death was quantified within three dimensional regions of interest (ROI) reconstructed from serial 'z-sections' (optical CLSM sections with reference to the z-axis) of the long edge of the rectangular blocks of articular cartilage. For z-sections acquired parallel to the z-axis (imaging all zones within the full thickness of cartilage), percentage cell death (PCD_{FT}) was quantified at 100μm intervals from the articular surface downwards within a ROI measuring 971 x 100 x 60μm^3 (x-y-z axes, respectively). For z- sections acquired perpendicular to the z-axis (imaging only the superficial zone below the articular surface), percentage cell death (PCD_{SZ}) was quantified within a ROI measuring 971 x 500 x 60μm^3 (x-y-z axes, respectively).

Results: The mean thickness of the articular cartilage "on" and "off" bone was 573.4 $\pm$ 25.3μm and 533.5 $\pm$ 23μm, respectively ($p=0.3$). PCD_{FT} suggested cell death was localised to the superficial zone ($\sim$ first 100μm from the articular surface) for Groups A, B and C at 2.5 hours and 7 days (analysis of variance, $p<0.05$ for all comparisons). PCD_{SZ} was similar for Groups A and B at 2.5 hours (controls, $p>0.05$, Tables 1 and 2).

Table 1. Increase in superficial zone chondrocyte death with excision of subchondral bone

Time	Group	PCD_{SZ} $\pm$ standard error
2.5 hours	Group A (off bone)	7.1±2.6
	Group B (on bone)	4.7±0.6
7 days	Group A (off bone)	25.8±3.6
	Group B (on bone)	6.4±1.3

With alternate day media changes (Table 1), PCD_{SZ} was significantly greater at 7 days for Group A compared to Group B ($p=0.003$). There was a significant increase in PCD_{SZ} from 2.5 hours to 7 days for Group A ($p=0.001$) but not for Group B ($p=0.2$). With no media changes (Table 2), PCD_{SZ} was significantly lower at 7 days for Group C compared to Group A ($p=0.001$), but similar to Group B ($p=0.2$). There was no significant increase in PCD_{SZ} for Group C from 2.5 hours to 7 days ($p=0.1$).

Conclusions: In bovine tissue, excision of subchondral bone from articular cartilage results in chondrocyte death at 7 days that occurs almost exclusively in the superficial zone. However, the presence of subchondral bone in the culture medium protects against chondrocyte death. These data suggest that subchondral bone may interact with articular cartilage via soluble mediator(s) that can influence chondrocyte survival.

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DYSFUNCTION OF MITOCHONDRIAL RESPIRATORY CHAIN IN NORMAL CHONDROCYTE. INFLAMMATION AND MATRIX DEGRADATION

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Purpose: Previously, we demonstrated that alterations of mitochondrial respiratory complexes III and V may contribute to the progression of chondrocyte inflammation, up-regulating the COX-2 enzyme but not the COX-1. However, the contribution of mitochondrial dysfunction in the degradation of extracellular matrix and the implication of stress inducing signals is not well defined. In this study, we have investigated the relationship between the dysfunction of mitochondrial respiratory chain (MRC) and the inflammatory response in human normal chondrocytes, the possible degradation of extracellular matrix, as well as the implication of calcium efflux signals, ROS and NF-κB in this process.

Methods: Human normal chondrocytes were isolated from cartilage obtained from autopsies without history of joint disease. Antimycin A (AA) and oligomycin were employed to inhibit the mitochondrial complexes III and V, respectively. N-acetyl cysteine (NAC), Ruthenium Red (RR) and BAY 117085 were employed as ROS, mitochondrial calcium exchange or NF-κB inhibitors, respectively. PGE_2 production was evaluated by ELISA. Flow cytometry was used to study the mitochondrial calcium exchange using Rhod 2-AM. Expressions of mRNA of MMPs were studied by real time PCR.

Results: Firstly, we assesed the production of the inflammatory mediator PGE_2 in normal chondrocytes in the presence of AA (40 μg/ml) and oligomycin (10μg/ml). AA and oligomycin increased PGE_2 after 24 hours of treatment (505.32±131.71 and 287.66±103.68 respectively vs basal 28.69±9.19 pg/50.000 cells, $p<0.01$). Secondly, pretreatment for 2 h with NAC 2mM or RR 25μM decreased the effect of AA up to 59.83±5.05% and 49.24±6.67%, respectively vs basal 100%, $p<0.01$. Values for oligomycin decreased up to 30.26±5.93% and 30.25±25.24% vs basal 100%, $p<0.01$. Thirdly, we examined whether NF-?B is required for this PGE_2 production. BAY 117085 (5 μM) reduced up to 23.50±5.26% the effect of AA and up to 35±8.48% the effect of oligomycin in the production of PGE_2 vs basal 100%, $p<0.01$. Finally, when calcium efflux was evaluated, we found that the dysfunction of complexes III and V, produced a release of calcium from mitochondria in a time-dependent manner. After 2 h of treatment with AA or oligomycin, levels of positive cells for mitochondrial calcium were 15.65±1.21% and 53.33±3.4% vs 61.3±1.69%, respectively, $p<0.01$. Responses to blockers of the mitochondrial calcium exchange indicated that ROS production was mitochondrial calcium depedent. Also, treatment with vitamin E, protected against AA and Oligomycin induced PGE_2. Moreover, as MMPs are enzymes related to extracellular matrix degradation, we studied the mRNA expression of MMP-1, -3 and -13. Oligomycin produced an increase of MMP-1 and -3 ratio (633.87 and 557.32 vs basal 1). In addition to this, we observed an up-regulation of mRNA of MMP-13 when the complex III didn't work properly (39.07 vs basal 1).

Conclusions: These results showed that a dysfunction of mitochondrial respiratory complexes III and V, induced an inflammatory and a degradation response in human normal chondrocytes.

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STUDY OF *IN VITRO* CONDITIONS PROMOTING HYPERTROPHIC DIFFERENTIATION OF OSTEOARTHRITIC ARTICULAR CHONDROCYTES

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Purpose: Hypertrophic differentiation of chondrocytes is an important feature in OA cartilage which is involved in extracellular matrix mineralization. This study aimed to develop a new culture model for studying chondrocyte hypertrophic differentiation *in vitro*

Methods: Articular OA chondrocytes were cultured for 28 days in monolayer or in alginate beads in different culture medium (1% Insulin Transferrin,Selenium and BSA, 2% Ultroser G or 10% Fetal Bovine Serum). DNA was quantified by fluorimetry. The expression of gene characteristics of chondrocyte phenotype (AGG, COL2A1, SOX9), pre-hypertrophic differentiation (ihh, PTH-R, PTHrP), hypertrophic differentiation (COL10A1, cbfa1), matrix mineralization (Ank, ENPP1, CILP, TG2, FXIIIA) or vascularization (VEGF) was evaluated by RT-PCR. Alkaline phosphatase

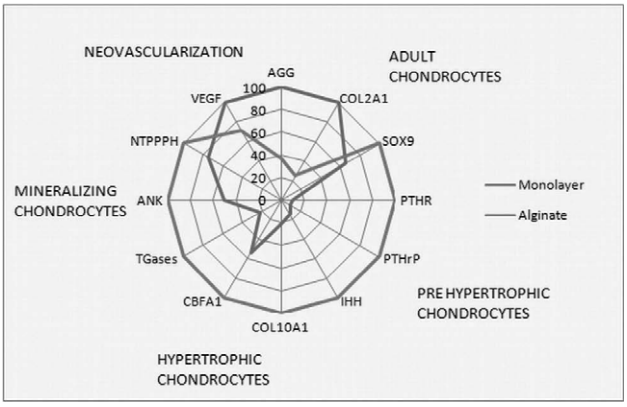

Figure 1. Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in 10% FBS media, n=3.

activity, transglutaminase activity and 5' phosphodiesterase activity of NTPPPH were quantified by specific enzymatic methods
Results: In 10% FBS, chondrocytes expressed significantly more hypertrophic (PTH-R, PTHrP, ihh, COL10A1 and cbfa1) and matrix mineralization genes (TG2, FXIIIA, CILP, ENPP1 and Ank) in alginate beads than in monolayer (Figure 1). Whatever the culture system (alginate beads or monolayer), chondrocytes cultured in 10% FBS expressed significantly more hypertrophic and matrix mineralization genes like COL10A1 than chondrocytes cultured in 1% ITS+ or 2% UG (Figure 2).

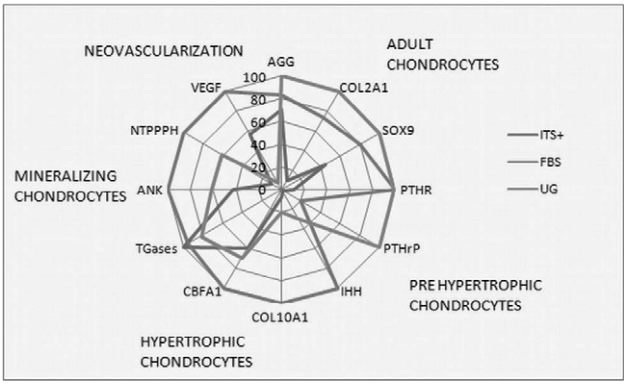

Figure 2. Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in alginate beads, n=3.

Conclusions: The expression of hypertrophic markers in articular chondrocytes is upregulated in fetal bovine serum (FBS) and alginate beads culture conditions, suggesting that cartilage neovascularization could be a factor of chondrocyte hypertrophic differentiation in OA cartilage

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MOLECULAR COMPARISON OF PRIMARY HUMAN CHONDROCYTES, IMMORTALIZED HUMAN CHONDROCYTES (T/C-28A2 AND C-28/I2), AND A HUMAN CHONDROSARCOMA CELL-LINE (SW1353) USING GLOBAL TRANSCRIPTIONAL PROFILING

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Purpose: Primary human chondrocytes are difficult to obtain, and their gene expression and response to cytokine stimuli are often inconsistent from donor to donor since factors such as disease severity and donor age are complicating variables. Therefore, immortalized chondrocyte-based cell lines are tempting alternatives for use in cell-based assays. However, these cell lines are phenotypically different from the primary chondrocytes (more fibroblast-like, minimal extracellular matrix secretion) and a limited molecular characterization has revealed some differences (Finger et al., 2003), yet no extensive analysis has been performed previously.
Methods: In order to investigate in detail the similarities and differences between primary chondrocytes and the chondrocytic cell lines T/C-28a2, C-28/I2 and SW1353, cells were treated in monolayer culture with or without IL-1 (1ng/ml) or TNF (10ng/ml) for 18 hours and global transcriptional changes were studied using Affymetrix gene chips. Focused gene expression was also analyzed using a TaqMan Low Density Array (TLDA) Immune Panel from Applied Biosystems, which measures the transcriptional levels of 90 immune- and inflammation-related genes simultaneously.
Results: Global profiling using correlation maps and hierarchical clustering indicated that transcriptional patterns in T/C-28a2, C-28/I2 and SW1353 are significantly different than in the primary chondrocytes. The transcriptional patterns of these samples clustered more closely to fibroblastic cell lines, such as BJ cells, than to the primary chondrocytes. The magnitude of transcriptional response to cytokines by primary chondrocytes was generally more than 10 fold greater than that of the immortalized cell lines. Transcriptional profiles of the chondrocyte cell lines T/C-28a2 and C-28/I2 were also significantly different from each other, and genes that were differentially expressed in the two cell lines were identified. Clustering analysis, Welch t-tests, as well as comparison of selected chondrocyte-specific gene expression patterns showed that the T/C-28a2 cell line is more similar to the human primary chondrocytes than the C-28/I2 line. Cytokine responsive gene sets common to all of the cells as well as gene sets unique to each cell type were identified for both IL-1 and TNF. Finally, TLDA analysis of immune related gene expression allowed detection of gene expression that was not measurable by gene chip analysis due to its lower sensitivity.
Conclusions: Our transcriptional analysis study using gene chip and TLDA showed that the immortalized chondrocytes (and SW1353 chondrosarcoma cells) that are frequently used in cartilage research are significantly different from primary chondrocytes. These results should be taken into consideration before choosing these cells as a substitute for primary chondrocytes in *in vitro* experiments.

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CORM-2 DOWN-REGULATES DEGRADATIVE ENZYMES AND ENHANCES EXTRACELLULAR MATRIX COMPONENTS IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: The articular chondrocyte is responsible for both matrix production and destruction. Excessive collagen II degradation is accompanied by loss of aggrecan in osteoarthritis (OA). Collagen II breakdown is mediated primarily by collagenases while aggrecan degradation is dependent on the activation of matrix metalloproteinases (MMPs) and aggrecanases. We have recently shown that the CO-releasing molecule tricarbonyldichlororuthenium(II) (CORM-2) inhibits aggrecan degradation in OA cartilage explants. The objective of this work was to investigate the effects of CORM-2 on extracellular matrix components and the mechanisms involved.
Methods: Cartilage specimens were obtained from 15 patients with diagnosis of advanced OA undergoing total knee joint re-