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