Under normoxia, IL-1β promotes chondrocyte dedifferentiation but IL-1β and NE effects under physiological hypoxic conditions are not yet investigated. Therefore, the aim of this study was to analyse the response of human articular OA chondrocytes to NE in the absence or presence of IL-1β depending on the oxygen concentration.
Methods: Articular cartilage samples were obtained from OA patients undergoing total knee replacement surgery. After isolation, cells were seeded in a density of 20.000 cells/cm^2 and cultured in a humidified atmosphere at 37°C and in 2% O_2 or 20% O_2, respectively. Cells were treated with different concentrations of NE (10^{-8} M, 10^{-6} M) and/or IL1-β (low dose: 0.5 ng/ml). Cell viability was determined in supernatants using an LDH assay. Adrenergic receptor (AR) profile of freshly isolated chondrocytes was analysed by PCR. At day 7, cell morphology was documented by phase contrast microscopy and sulphated proteoglycans (sGAG) in the extracellular matrix were visualized via DMMB staining. Quantitative PCR was preformed to analyse Col1A1, Col2A1, COMP, Sox-9 expression.
Results: Cell viability was not affected by any treatment. Freshly isolated chondrocytes expressed α2A-, α2C- and β2-AR. At day 7, untreated chondrocytes showed the characteristic morphological shift towards a fibroblast-like shape independent on oxygen concentration. NE alone did not affect cell morphology, but in combination with IL-1β markedly accelerated this shift indicating dedifferentiation. Similarly, we observed moderate DMMB staining in untreated and NE treated cells, while treatment with IL-1β alone and in combination with NE strongly decreased sGAGs.Under normoxic conditions, untreated cells expressed slightly increased Col1A1, decreased Sox-9, unchanged Col2A1, and slightly decreased COMP levels (Fig. 1) in agreement with previous studies. Hypoxia decelerated the dedifferentiation process with stable Sox-9 and COMP levels and markedly increased Col2A1 levels, only Col1A1 expression increased significantly (Fig. 1). Treatment with IL-1β alone did not induce further changes in Col1A1 expression, but caused a slight Sox-9 and a highly significant Col2A1 and COMP decrease in both normoxic and hypoxic cultures (Fig. 1). NE alone did not influence gene expression levels and was also not able to modulate IL-1β-mediated effects neither under hypoxic nor under normoxic conditions.
Conclusions: This study shows that articular chondrocyte dedifferentiation is decelerated under hypoxic conditions and that IL-1β completely reversed this effect. NE was not able to exhibit any modulating effect. Thus, low-grade inflammation exerts a dominant effect during OA pathogenesis and should be targeted early in OA therapy. Analysing the major sources of IL-1β in the joint and further molecular effects of mild inflammation during OA pathogenesis will be the focus of our future work.

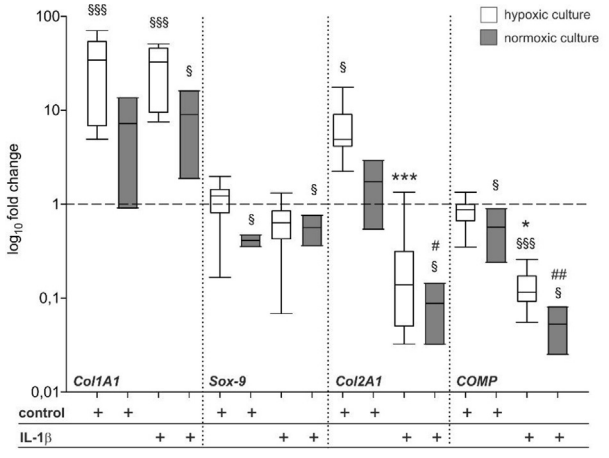

Fig.1 Gene expression levels in human articular chondrocytes.
Expression of Col1A1, Sox-9, Col2A1, and COMP genes in human articular chondrocyte cultures at day 7 of monolayer dedifferentiation under normoxia, hypoxia, and in the absence or presence of IL-1β.
Data are presented as box plots with the 10th, 25th, 50th (median), 75th, and 90th percentiles. Values are demonstrated relative to gene expression levels in chondrocytes at day 0 (day 0 levels = 1, dashed line). Significant p-values against chondrocytes at day0 are presented as § (p≤0.05) or §§§ (p≤0.001), against hypoxic control as "*" (p≤0.05) or "***" (p≤0.001) and against normoxic control as "#" (p≤0.05) or ##" (p≤0.01).

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HYALURONIC ACID & NATRIUM SUCCINATE IN TREATMENT OF EXPERIMENTAL OSTEOARTHRITIS

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Purpose: To determine the influence of the combination of HA & sodium succinate on knee OA in experiment.
Methods: Experimental study was performed on 32 rabbits of the Chinchilla breed with OA of the knee joint, induced by transchondral injury (2 mm in diameter & 3 mm in depth) - in patellar surface of the femur. To assess the effectiveness of treatment, all animals were divided into 2 groups. In group 1 16 animals received 5 intra-articular injections of a 1.1% solution of HA & sodium succinate in a dose of 0.2 ml every 7 days. Rabbits of group 2 (control group, 16 animals), received 5 intra-articular injections of 1.1% solution of the non-stabilized HA. To assess the results, the morphological study of the cartilage (from injured and non-injured joints) was performed at day 45.
Results: The morphology of the cartilage from injured joints demonstrated clear signs of post-traumatic OA with the small foci of chondrocytes & fibrous regenerative cartilage-like tissue at points of direct injury; significant degenerative & necrotic changes around cartilage wounds. The use of combination of 1.1% solution of HA & sodium succinate for treatment (group 1) was accompanied with smaller area of necrotic & degenerative changes of cartilage & more active formation of cartilaginous regenerate in the injured zone in comparison with the use of a 1.1% solution of the HA alone (group 2), which can be explained by the additional anti-hypoxic effects of sodium succinate & possible stimulation of chondrocytes.
Conclusions: Use of the combination of HA & sodium succinate in experimental post-traumatic knee OA treatment has an advantage over the use of HA alone.

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DIFFERENT REGULATORY ROLES OF TNF-α AND IL-6 IN OVARIECTOMY INDUCED BONE LOSS

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Purpose: An imbalance in bone formation relative to bone resorption and paralleled bone loss induced by ovariectomy (OVX) have been associated with elevated levels of pro-inflammatory cytokines. However, it is unclear what regulatory roles these cytokines act in mediating OVX-induced bone loss, and whether the adverse effects of these cytokines differ on bone metabolism. Here, we examined the effects of tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) gene knockout in preserving the bone loss induced by ovariectomy (OVX) and the mechanisms involved in bone metabolism.
Methods: Twenty Female wild-type (WT), *TNFα* knockout (TNFα$^{-/-}$) or *IL6* knockout (IL6$^{-/-}$) mice aged 12 weeks were sham-operated (SHAM) or subjected to OVX and euthanized after 4 weeks. Bone mass and skeletal microarchitecture were determined using micro-CT. Bone marrow stromal cells (BMSCs) from all three groups (WT, TNFα$^{-/-}$ and IL6$^{-/-}$) were induced to differentiate into osteoblasts or osteoclasts, and treated with 17-β-Estradiol. Bone metabolism was assessed by histological analysis, serum analyses and qRT-PCR.
Results: OVX significantly increased the body weight only in WT mice and decreased the uterine weight in all three genotypes. The high bone turnover induced by OVX was largely repaired by TNFα and IL6 gene knockout. The ratio of femoral trabecular bone volume to tissue volume, trabecular number and trabecular thickness were significantly decreased in WT mice subjected to OVX, but increased in TNFα$^{-/-}$ mice (1.62, 1.34, 0.27-fold respectively) and IL6$^{-/-}$ mice (1.34, 0.80, 0.22-fold respectively). Furthermore, we observed a 29.6% increase in the trabecular number in TNFα$^{-/-}$ mice when compared to the IL6$^{-/-}$ mice. Both, TNFα$^{-/-}$ and IL6$^{-/-}$ BMSCs exhibited decreased numbers of TRAP-positive cells and an increase in ALP positive cells, with or without E2 treatment. While the knockout of *TNFα* or *IL6* significantly up-regulated mRNA expressions of osteoblast related genes (*Runx2* and *Col1a1*) and down-regulated osteoclast related mRNA for *TRAP, MMP9* and *CTSK in vivo* and *in vitro*, *TNFα* knockout appeared to have roles beyond *IL6* knockout in upregulating *Col1a1* mRNA expression and downregulating mRNA expressions of WNT-related genes (*DKK1* and *Sost*) and TNF-related activation-induced genes (*TRAF6*).
Conclusions: While TNFα and IL6 both had an important role in mediating OVX-induced bone loss, TNFα had a potentially stronger