

NSAIDs and NSAID-specific inhibition of prostaglandin synthesis were determined by colorimetric activity assays and EIAs. Gene- and protein expression analyses were employed to determine the effects on chondrogenic extracellular matrix formation.

**Results:** COX-specificity of the NSAIDs was confirmed and for subsequent experiments NSAID concentrations were selected that resulted in similar degrees of COX-1 or COX-2 inhibition. COX-1 specific NSAIDs inhibited Col2a1 and Col10a1 expression. Inhibition of COX-2 resulted in substantial decrease of Col10a1 expression, while Col2a1 remained unaffected. To explain this difference we determined the expression patterns of both COX enzymes as well as specific prostaglandin synthesis during differentiation. COX-1 is upregulated during late chondrogenic differentiation, whereas COX-2 is briefly expressed early in differentiation and increases again late in differentiation. PGD<sub>2</sub> and PGE<sub>2</sub> faithfully followed the COX-2 expression pattern, whereas PGF<sub>2a</sub> and TxB<sub>2</sub> levels remained stably low. Furthermore, COX-2 inhibition resulted in decreased levels of all tested PGs, whereas COX-1 inhibition inhibited synthesis of all PGs, except for PGD<sub>2</sub>, which was increased.

**Conclusions:** Our findings point towards a differential role for COX-enzymes and PG-production in chondrogenic differentiation. Ongoing research is focussing on further elucidating the functional partition of cyclooxygenases and specific prostaglandin production. Our data add to the pallet of possibilities for improving the collagen quality of cartilage constructs for better outcome of cartilage regenerative approaches.

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### BMP-2 AND BMP-7: DIFFERENTIAL REGULATION OF CHONDROGENIC DIFFERENTIATION

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**Purpose:** Bone morphogenic protein (BMP)-2 and BMP-7 are known to induce (ectopic) bone formation. The recombinant human versions of these proteins are clinically approved and used to promote osteogenesis at sites of poor fracture healing (non-unions) and as additional factors to enhance integration of bone grafts. Recently, molecular studies have shown that BMPs are also able to regulate the chondrogenic regenerative potential. However, the differential influence of different BMPs on the chondrogenesis of progenitor cells is unknown.

**Methods:** Equimolar concentrations of BMP-2 and BMP-7 were added from the initiation of chondrogenic differentiation of ATDC5 and hBMSCs onward. Chondrogenic markers (Col2a1, Sox9), chondrocyte hypertrophic markers (Col10a1, RunX2) and endochondral regulatory mediators were determined by gene- and protein expression analysis.

**Results:** Supplementing BMP-7 to chondrogenically differentiating ATDC5 cells resulted in an overall increased Col2a1 expression and decreased chondrocyte hypertrophy. In contrast, BMP-2 dose-dependently increased chondrocyte hypertrophic markers Col10a1 and RunX2, whereas Col2a1 levels did not differ from control conditions. This profound differential action of BMP-2 and BMP-7 on ATDC5 endochondral differentiation could also be confirmed in hBMSC endochondral differentiation. Interestingly, we found that COX-2 was specifically induced by BMP-2 and not by BMP-7 possibly explaining the differential hypertrophic actions these BMPs.

**Conclusions:** BMP-2 and BMP-7 show differential effects on the chondrogenic outcome of differentiating chondroprogenitor cells: BMP-2 acts a specific inducer of chondrocyte hypertrophy, while BMP-7 appears to increase or maintain chondrogenic potential. We are now establishing the underlying mechanism which explains this striking differential effect. Our results may provide novel leads to optimize cartilage regenerative techniques on a differential BMP basis.

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### ESTABLISHMENT OF NOVEL THREE-DIMENSIONAL CULTURE SYSTEM FOR HUMAN CHONDROCYTES USING MICRO-SPACE PLATE

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**Purpose:** It has been reported that monolayer culture of human articular chondrocytes results in dedifferentiation and loss of chondrogenic property. Therefore, the dedifferentiation is a big problem when the influence

of cytokines and drugs were evaluated. In order to solve this problem, we performed a novel three-dimensional culture system for human chondrocytes using micro-space plates compared with monolayer culture.

**Methods:** The micro-space plate has regularly arranged 200 mm×100 mm×50 mm square compartments on its surface. Human articular chondrocytes were cultured in flat plates or micro-space plates for 1 week. After culture, the level of collagen 1A1 (COL1A1) and aggrecan mRNA expressions were measured by real-time PCR, and cartilage matrix production was assessed by alcian blue staining. Furthermore, chondrocytes were cultured with IL-1β, IL-6+soluble IL-6 receptor (sIL-6R) in the presence or absence of high molecular hyaluronic acid (HA) for additional 24 h and then the production of MMP-3 in cell supernatant was measured by ELISA.

**Results:** At the same passage of cells, human articular chondrocytes grown on micro-space plates in clusters after 5-7 days' culture, whereas cells grown on flat plates in fibroblast-like morphology. In three-dimensional culture, the mRNA expression levels of COL1A1 and aggrecan were higher than those in monolayer culture. And the cartilage matrix production in three-dimensional culture was higher than that in monolayer culture. Moreover, the production of MMP-3 induced by IL-1β and IL-6+sIL-6R was higher in three-dimensional culture than in monolayer culture. Inhibitory activity of HA on MMP-3 production was similar between three-dimensional culture and monolayer culture. Over the subsequent passage, the expression levels of COL1A1 and aggrecan mRNA and MMP-3 production by IL-1β and IL-6+sIL-6R gradually decreased in monolayer culture, but they remained at higher levels in three-dimensional culture.

**Conclusions:** In conclusion, the present study demonstrated that the novel three-dimensional culture using micro-space plates maintains chondrogenic properties. Three-dimensional culture using micro-space plates is a useful tool for chondrocyte culture.

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### A STUDY OF THE LOCAL RAS IN ATDC5 CHONDROPROGENITOR CELLS AND EPIPHYSEAL PLATES OF MICE

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**Purpose:** Recently, the local renin-angiotensin system (RAS) has attracted many researchers in many pathophysiological issues. In Orthopedics, expression of local RAS was found in bone tissues, fracture callus and arthritic synovium. The purpose of this study is to reveal immunohistological localization of the RAS components in the epiphyseal plates of mice and to analyze function of the local RAS in the processes of hypertrophic differentiation using ATDC5 chondroprogenitor cells.

**Methods:** The epiphyseal plates of 8-week-old mice was immunostained with antibodies to angiotensinogen, angiotensinogen converting enzyme 1 (ACE1), angiotensinII type 1 receptor (AT1R) and angiotensinII type 2 receptor (AT2R). We cultured ATDC5 in long term and evaluated the expression of angiotensinogen, ACE1, AT1R, AT2R and type 2 collagen (COL2) by real-time PCR and Western blot analysis.

**Results:** In the epiphyseal plates of mice, angiotensinogen and AT1R expressed in the resting chondrocytes, the proliferative chondrocytes and hypertrophic chondrocytes; however, ACE1 and AT2R expressed only in the hypertrophic chondrocytes. In ATDC5 chondroprogenitor cells, the local RAS components expressed both in proliferative and hypertrophic differentiating stages.

**Conclusions:** The local RAS expresses in the epiphyseal plates of mice and might play an important role in the process of hypertrophic differentiation.

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### ESTROGEN'S STIMULATING EFFECT ON ATDC5

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**Purpose:** The prevalence of knee osteoarthritis between Menopausal woman increase sharply. There are many studies indicate that the level of serum estrogen is associated with the morbidity of knee OA tightly. This