# ARTICULAR MOTION STIMULATES THE PRG4 GENE EXPRESSION IN CHONDROCYTES FROM BOTH SUPERFICIAL AND DEEP ZONE CARTILAGE

+*Grad, S; *Li, Z; **Wimmer, MA; *Alini, M
+*Biomaterials and Tissue Engineering Program, AO Research Institute, Davos, Switzerland
sibylle.grad@aofoundation.org

## INTRODUCTION:

In articular cartilage, the proteoglycan 4 (PRG4) gene is expressed predominantly by chondrocytes from the superficial zone. Accordingly, its product has also been named superficial zone protein (SZP), and it can be considered as a marker of superficial zone chondrocytes [1]. At the articular surface, PRG4 functions as an effective boundary lubricant, prevents cell attachment and protects underlying cells. We have identified that distinct mechanical stimuli, in particular articular surface motion, promotes the gene expression and synthesis of PRG4 in chondrocytes isolated from full thickness bovine metacarpal joint cartilage [2], suggesting that chondrocytes adapt to their mechanical environment. Full thickness articular cartilage comprises a heterogeneous cell population, and differences in metabolic activity and response to mechanical and growth factor stimulation of subpopulations have been described. Therefore, it is unknown whether chondrocyte subpopulations respond in a similar manner to articular surface motion or whether intrinsic differences exist in their responsiveness. This study specifically examined the effect of applied articular motion on the gene expression of PRG4 in chondrocytes isolated from the superficial and deep zone of bovine knee joint cartilage.

## METHODS:

Superficial zone cartilage was harvested from the patella, the femoral condyle and femoral groove from 2-4 months old calves by careful abrasion of the surface using a scalpel blade. Deep zone cartilage was harvested from the lower third of the remaining tissue of the femoral groove. Chondrocytes were isolated by sequential pronase and collagenase digestion, suspended in fibrin solution and seeded into resorbable porous polyurethane scaffolds [3] (8 mm diameter, 4 mm thick) at a density of 10 x $10^6$ per scaffold. Alternatively, the cells were plated in culture dishes and used for experiments after the first passage. Cell-scaffold constructs were kept in static culture for 5 days in DMEM supplemented with 10% FCS, nonessential amino acids, 50 µg/ml ascorbate and 40 µg/ml L-proline. Then they were exposed to mechanical perturbation using a new bioreactor that provides joint specific biomechanical stimuli (complex shear force patterns on the surface of cell-seeded scaffold) [4]. Cyclic axial compression (0.4 mm offset, 0.4 mm sinusoidal strain, 0.1 Hz) was performed by a ceramic hip ball 32 mm in diameter, with or without articular surface motion. During the latter, oscillation of the ball over the construct surface was applied (±25°, 0.1 Hz). Two one-hour loading periods were performed daily over three consecutive days. Unloaded constructs served as controls. After loading, constructs were horizontally cut into two sections (top and bottom). Total RNA was extracted from the cells of each section and standard real-time RT-PCR was carried out on an Applied Biosystems 7500 PCR System using primers and probe for bovine PRG4 [2] and 18S ribosomal RNA as endogenous control [5]. Gene expression levels were normalized to the basis level of the respective superficial zone chondrocytes before seeding them into the scaffolds (day 0). Results from three independent experiments, each run in duplicates, are presented. ANOVA was performed using STAT-VIEW, with $\alpha$=0.05.

## RESULTS:

The basic PRG4 gene expression level of isolated superficial zone chondrocytes exceeded the expression of deep zone cells by approximately 700 times (Fig. 1, day 0 values). This is consistent with previous reports and confirms the different phenotypes of the cells in the harvested tissue sections. Culture in 3D scaffolds and 6 one-hour periods of dynamic compression did not alter the PRG4 mRNA expression level of superficial zone chondrocytes. However, applied articular surface motion increased the PRG4 expression of these cells, with a similar response in both the top and bottom sections of the constructs (Fig. 1, S cells). In chondrocytes extracted from the deep zone, culture in 3D scaffolds significantly increased the PRG4 mRNA expression, which was further enhanced by compression and superimposed surface motion only in the top section of the constructs. Independent of mechanical stimulation, the PRG4 mRNA levels appeared to be higher in the top sections than in the bottom sections in all deep zone constructs (Fig. 1, D cells).

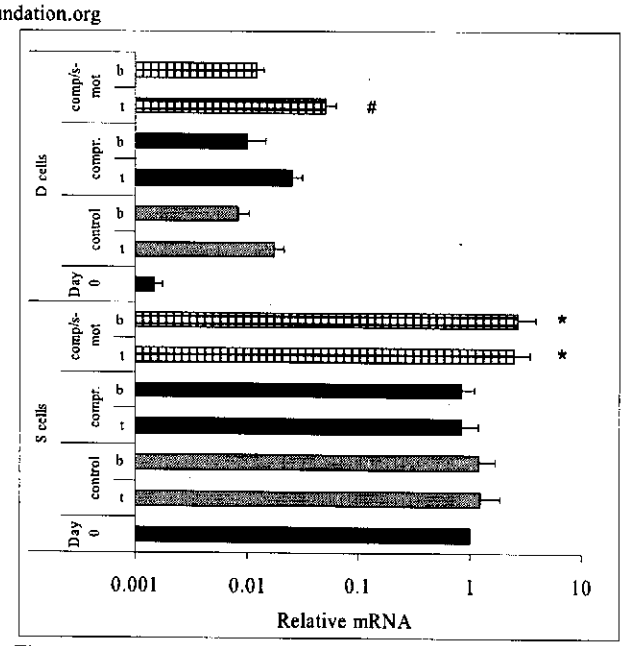


**Fig. 1.** PRG4 mRNA expression of chondrocytes isolated from superficial (S cells) and deep (D cells) zone cartilage and cultured in porous scaffolds. Top (t) and bottom (b) sections of unloaded constructs (control) and constructs exposed to cyclic axial compression without (compr.) or with (comp/s-mot) superimposed surface motion were analysed separately. mRNA levels are expressed relative to the level of the respective S cells at day 0 of culture. Mean + sem, n=6; *p<0.05 vs. S cell compression only sections; #p<0.05 vs. all other D cell sections.

## DISCUSSION:

Three-dimensional culture up-regulated the PRG4 expression in deep, but not in superficial zone cells, suggesting that while S cells maintain their phenotype, D cells react to changes in their environment towards the conditions at the articular surface (e.g. increased nutrient and $O_2$ supply). Interestingly, the up-regulation was more pronounced in the upper compared to the lower sections of the tested constructs, strengthening the hypothesis of an environmental influence (nutrients, $O_2$). In addition, articular surface motion enhanced the PRG4 expression of both S and D cells, confirming the positive effect of biomechanical stimuli [2]. The data provide evidence that different chondrocyte subpopulations can be induced to enhance their PRG4 expression. Although the expression level of the stimulated D cells was still lower than the basal level of the S cells, it increased up to 35 times in only 8 days of culture. Prolonged culture in an optimised chemical and mechanical environment may further stimulate the PRG4 expression up to the level of S cells. This finding may be of importance for tissue engineering approaches, where generally heterogeneous cell populations are applied. Furthermore, it may be speculated that after injury of the cartilage surface, under the appropriate biological and mechanical stimuli, cells of the underlying tissue accept the S cell phenotype and produce the PRG4 molecule with its essential functions for joint articulation.

## REFERENCES:

[1] Schumacher BL et al., *JOR* 17:110, 1999. [2] Grad S et al., *Tissue Eng.* 11(1-2):249, 2005. [3] Gorna K et al., *J. Biomed. Mater. Res.* 60:592, 2002. [4] Wimmer MA et al., *Tissue Eng.* 10(9-10):1436, 2004. [5] Lee CR et al., *Anal. Biochem.* 341:372, 2005.

## ACKNOWLEDGEMENT:

Partial support by the Swiss National Science Foundation.

** Dept. of Orthopedics, Rush University Medical Center, Chicago, IL