

# Notochordal cell-secreted factors stimulate matrix production by canine nucleus pulposus cells and bone marrow derived stromal cells

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**GP40****NOTOCHORDAL CELL-SECRETED FACTORS STIMULATE MATRIX PRODUCTION BY CANINE NUCLEUS PULPOSUS CELLS AND BONE MARROW DERIVED STROMAL CELLS**

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**INTRODUCTION:** Intervertebral disc degeneration is characterized by failure to maintain a healthy matrix. Recently, bone marrow derived stromal cells (MSCs) have been proposed to replenish the decreasing number of nucleus pulposus cells (NPCs) in the disc. Bioactive factors, secreted by notochordal cells (NCs) into conditioned medium (NCCM) have also been reported to stimulate proteoglycan production. Thus, we assessed, in a canine in vitro model, the stimulatory effects of NCCM on NPCs, MSCs and their mixture (NPCs+MSCs), as well as the effect of MSCs on NPCs alone.

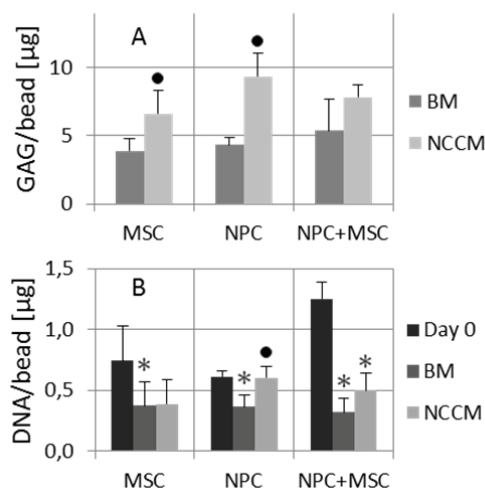
**METHODS:** MSCs and NPCs were harvested from chondrodystrophic (CD) dogs. NCCM was produced from NP tissue of non-CD dogs. MSCs or NPCs alone (3 million cells/ml) and NPCs+MSCs (6 million cells/ml; 1:1) were cultured for 4 weeks in 1.2% alginate beads and received base medium (BM, high glucose DMEM + 5% stripped FCS + 1% pen/strep) or NCCM (+ 5% stripped FCS + 1% pen/strep). Beads were assessed for GAG and DNA contents by biochemical assays, GAG deposition by Alcian Blue staining, and gene expression (collagen 2, aggrecan, and SOX9) with RT-qPCR.

**RESULTS:** GAG content increased in NCCM single cell groups compared to BM (Fig. 1A), whereas it did not with addition of MSCs to NPCs. Alcian Blue staining confirmed these

findings. DNA content decreased in time for all BM groups (Fig. 1B), and did not change for NPCs cultured in NCCM. At day 28, collagen 2, aggrecan, and SOX9 gene expression increased in NCCM treated NPCs and NPC+MSCs compared to day 0 and BM, whereas no differences were observed for NCCM treated MSCs.

**DISCUSSION:** NCCM induced increased NPC GAG production and directed NPCs towards a healthier phenotype. NCCM also stimulate MSCs, but combining NPCs+MSCs did not have an additive effect. The use of NC-secreted factors is promising, and identification of these factors is of interest.

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**Figure 1:** A) GAG per bead content at day 28 and B) DNA per bead content at day 0 and day 28. •  $p < 0.05$  compared to BM, \*  $p < 0.05$  compared to Day 0.

**GP41****INCREASED LEVELS OF NERVE GROWTH FACTOR AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN HUMAN HERNIATED INTERVERTEBRAL DISCS**

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