

Effect of TGF β ₁, BMP-2 and hydraulic pressure on chondrogenic differentiation of bovine bone marrow mesenchymal stromal cells androcytes

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INTRODUCTION: Bioactive factors such as TGF β ₁ and BMP-2 as well as mechanical factors i.e. compressive loading and hydraulic pressure, have been shown to induce and/or modulate chondrogenesis of bone marrow derived mesenchymal stromal cells (BMSCs). Although TGF β ₁ and BMP-2 belong to the same superfamily of ligands, they bind to different receptors (TGF β receptor and BMP-2 receptor, respectively) which lead to activation of different receptor regulated-SMAD molecules. More recently, Huang et al [1] have shown that cyclic compressive loading leads to up-regulation of endogenous TGF β gene expression as well as upregulation of type I and type II TGF β receptors. Hence, these factors are intracellularly transduced through different mechanisms and it is hypothesized that TGF β ₁, BMP-2 and hydraulic pressure may act synergistically on chondrogenic differentiation of BMSCs.

METHODS: BMSCs were isolated from three calves. After expansion, aggregates of BMSCs were cultured under serum free conditions in the presence of 10 ng/ml TGF β ₁, 50 ng/ml BMP-2 or both. Half of the samples were loaded for 4 hours per day with 0.5 to 3 MPa cyclic hydraulic pressure at 1 Hz. After 14 days of culture/loading, gene expression of collagen type I and II, aggrecan and Sox 9 was assessed. DNA as well as glycosaminoglycan (GAG) content of the pellets were analysed relative to day 1.

RESULTS Neither pressure nor BMP-2 had an influence on gene expression or GAG/DNA content. However, cells responded to the presence of TGF β ₁ with an up-regulation of chondrogenic genes and GAG/DNA of the aggregates increased compared to controls demonstrating the cells ability to respond to external stimuli (figure 1).

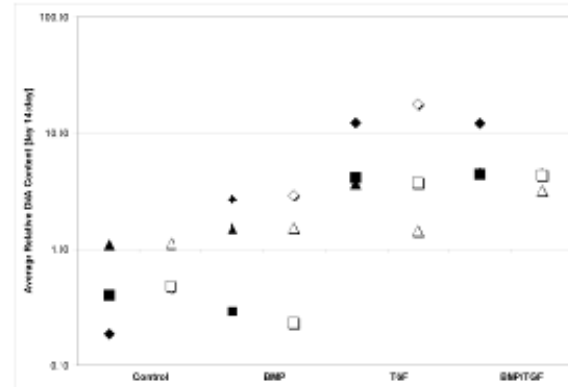


Fig. 1: GAG/DNA content of bovine BMSCs at day 14 relative to control day 1; solid symbol represent unloaded samples, unfilled symbols loaded samples; (n=3); logarithmic scale.

DISCUSSION & CONCLUSIONS: The upregulation of chondrogenic genes in the presence of TGF β ₁ confirms that cells within our system were able to respond to an external stimulus. BMP-2 and hydraulic pressure were neither able to induce nor modulate chondrogenesis in contrast to previous work by others. However, the effect of growth factors is dependent on many factors such concentration and duration of exposure, cell type, cell source, culture system and species of donor.

Several studies have shown only limited to no induction of chondrogenesis, if hydraulic pressure only was used. On the other hand compressive loading lead to an upregulation of chondrogenic genes in BMSCs even in the absence of TGF β . This may indicate a higher sensitivity of BMSC to compressive loading than to hydraulic pressure. The involvement of the TGF β pathway in mechanotransduction of compressive loading has been demonstrated [1]. To our knowledge the mechanotransduction of hydrostatic pressure in BMSCs has not been investigated yet. Differences in how mechanical stimulus is transduced might explain the differences between studies.

REFERENCES: ¹ C.Y Huang, P.M. Reuben, and H.S. Cheung (2004) *Stem Cells* **23**(8):1113-1121.