

MECHANICAL REGULATION OF CARTILAGE MATRIX PROTEINS AND ANGIOGENIC FACTORS BY MECHANICAL STRESS

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INTRODUCTION: Endochondral ossification involves a complex series of events including chondrocyte differentiation towards hypertrophy, calcification of the matrix, vascular invasion and the deposition of bone. The rate has been suggested to be influenced by intermittent patterns of shear/tension and hydrostatic stress¹. Single-phase continuum models of skeletal development show areas of high shear stress in secondary ossific nuclei of epiphyses and suggests that vascular invasion is inhibited in areas of high hydrostatic pressure². The goal of this study is to examine the effect of hydrostatic pressure and tension on the expression of cartilage matrix proteins and angiogenic factors.

METHODS: Primary chondrocytes were isolated from 6 calf bovine humeral heads cartilage using a sequential pronase/collagenase digestion. Cartilage from each shoulder was digested separately. The chondrocytes were suspended in a 2% alginate solution containing 4×10^6 cells/ml and were polymerised in cylinders for hydrostatic pressure and rectangular beams for tensile experiments. Struts of polyethylene were embedded in both ends of the rectangular beam for attachment to the tensile apparatus. The gels were cultured for five days and subjected to 3 additional days of tension, compression or pressure. Labview software was used to control movement of the 3 microtesting systems. All 3 loading modes were performed at 0.5 Hz, 3 hrs/day for 3 days. Alginate specimens were solubilised after the third day of loading and RNA was isolated using an RNeasy kit (Qiagen). RNA was reverse transcribed using the Gene Amp RNA kit (Perkin-Elmer). TAQman probe and primer sets were designed using the Primer Express software. Cartilage matrix proteins examined included collagen 1, 2, and 10, aggrecan, COMP, and superficial zone protein. Factors associated with angiogenesis during endochondral ossification include connective tissue growth factor (CTGF), MMP13, TIMP-1, and vascular endothelial growth factor (VEGF). PCR reactions were run on an ABI Prism 7700 Sequence Detection System (Applied Biosystems) using 10ng cDNA/reaction and a primer and probe concentration of 900 nM and 300 nM, respectively.

RESULTS: The largest change in gene expression resulted from uniaxial tension and compression. These loading modes caused an upregulation in the expression of collagen 10, COMP, and superficial

zone protein. Uniaxial tension also significantly upregulated the expression of CTGF, MMP-13 and down-regulated the expression of TIMP-1. Hydrostatic pressure had the chondroprotective effect of down regulating MMP-13 expression and collagen 1 expression.

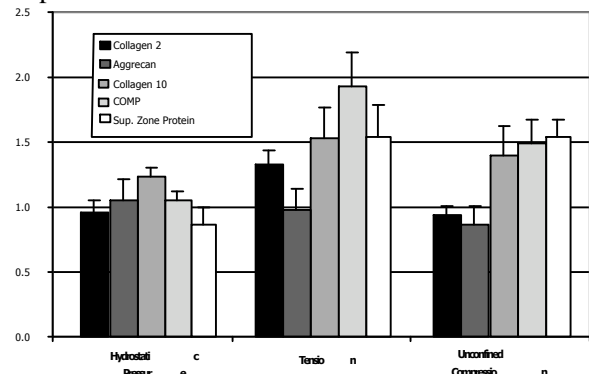


Fig. 1: Effect of mechanical loading on synthesis of cartilage matrix proteins (n=6).

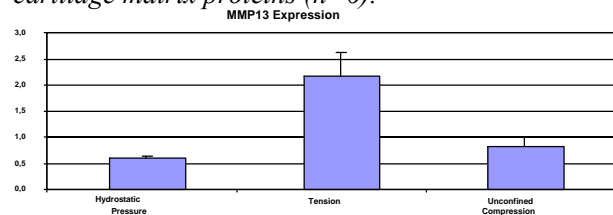


Fig. 2: Tension up-regulates MMP-13 expression while hydrostatic pressure down-regulates its expression.

DISCUSSION & CONCLUSIONS: Hydrostatic pressure and uniaxial tension have opposing effects on the expression of cartilage gene expression. Uniaxial tension upregulated expression of matrix proteins involved in hypertrophy (type X collagen) and vascular invasion (MMP-13, CTGF) and down-regulated expression of chondroprotective genes such as TIMP-1. The effect of hydrostatic pressure was to inhibit the expression of MMP-13 and to maintain the chondrogenic phenotype by down-regulating expression of type I collagen. Interestingly, the gene expression pattern of the two large-strain loading modes, uniaxial tension and compression, were quite similar. The expression of superficial zone protein was upregulated by both high strain modes, a finding which is consistent with its in vivo pattern of expression in the highly strained superficial layers.

REFERENCES: ¹ D. Carter, M. Wong (1988) *J. Orthop. Res.*, 6:804-816, 1988 ² D. Carter, G. Beaupre, (2001) *Skeletal Form and Function*, Cambridge University Press.

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