Introduction: The mechanical environment is an important factor that maintains articular cartilage in a healthy state. Mechanical signals generated under normal physiological loading conditions will activate mechanotransduction pathways, and drive biochemical events that regulate chondrocyte function and activity [1]. However, under abnormal mechanical loading, the homeostatic balance between the catabolic and anabolic events is disturbed, leading to cartilage breakdown and the development of osteoarthritis (OA) [1]. Interleukin-1β (IL-1β) acts as the key mediator of these processes and stimulates the production of nitric oxide (NO) and prostaglandin E₂ (PGE₂), via the inducible nitric oxide synthase (iNOS) and cyclo-oxygenase (COX-2) enzymes and members of the mitogen activated protein kinase (MAPK) family [2]. We have previously shown that application of physiological levels of dynamic compression abolished the IL-1β induced production of NO and PGE₂ in bovine and human chondrocytes cultured in agarose constructs [3–4]. Accordingly, the present study examines the effects of IL-1β and dynamic compression on the expression levels of iNOS and COX-2 and the involvement of p38 MAPK.

Materials and Methods: Bovine chondrocytes were seeded in 3% agarose (4 x 10⁶ cells.ml⁻¹) and equilibrated in culture for 24 hours [3–4]. Chondrocyte / agarose constructs were cultured under free-swelling conditions with 0 or 10 ng.ml⁻¹ IL-1β and / or 10 μM SB203580 (p38 MAPK inhibitor) for up to 48 hours. Using a well-characterised bioreactor system [3–4], constructs were subjected to 0-15% dynamic compression at 1 Hz frequency for 6, 12 and 48 hours under selected treatments. Total RNA was isolated and reverse transcribed using oligo(dT) primers and the Stratascript™ reverse transcriptase. Real-time quantitative PCR assays coupled with molecular beacons were performed with cDNA, Brilliant® QT-PCR Master Mix, primer pairs and analysed on the MX3000P instrument. The ratio of the relative expression levels of iNOS and COX-2 signals was accomplished by normalizing each target to the reference gene, GAPDH and to the calibrator sample, by a comparative cycle threshold (Ct) approach. Additionally, real-time PCR efficiencies were incorporated into the analysis. 2-way ANOVA with post-hoc Bonferroni corrected t-tests were used to examine differences, with significance at a 5% level.

Results: In the absence of the cytokine, iNOS and COX-2 expression did not significantly change over a period of 48 hours (Fig. 1).

Discussion: Dynamic compression reduced iNOS expression and co-stimulation with both dynamic compression and the p38 MAPK inhibitor, SB203580, resulted in a further reduction, suggesting that dynamic compression targets the p38 MAPK dependent pathway. COX-2 induction by the cytokine appears to be primarily mediated by a p38 MAPK dependent pathway that is highly susceptible to either the p38 MAPK inhibitor or dynamic compression. At present, we are using the bioreactor system as a tool to further investigate the mechanosensitive pathways. This methodology will help to play a key role in the identification of appropriate pharmacological agents for cartilage repair and provides a clinical rationale for promoting the benefits of controlled physical activity to treat OA.


Acknowledgements: This study was supported by The Wellcome Trust (073972).