INTRODUCTION:
Osteoarthritis is a notoriously complex disease and there are likely to be multiple processes, underlying its cause and determining its progression. Both molecular and mechanical factors will affect tissue homeostasis resulting in pathways which converge and cross talk with several routes. One such signalling pathway involves the ubiquitous transcription factors of the NFκB family which are known to be activated by IL-1β. In chondrocytes, NFκB activity is regulated by IκB-α and plays a role in the transcription of a number of inflammatory genes encoding IL-1β, iNOS and COX-2, influenced by mechanical loading [1]. We previously demonstrated a downregulation of iNOS and COX-2 expression by dynamic compression in chondrocyte/agarose constructs cultured with IL-1β but did not examine the involvement of NFκB [2]. Thus, the suppression of the inflammatory genes by mechanical loading may occur at the transcriptional level, implicating both mechanical and NFκB signals as key regulators in normal and pro-inflammatory cascades. This study therefore explored the potential of IL-1β and dynamic compression to influence NFκB activation and IκB-α expression in chondrocytes cultured in agarose constructs.

METHODS:
Chondrocytes were isolated from the bovine joint, seeded in 3% agarose type VII (4 x 10^5 cells.ml^-1) and equilibrated in culture for 24 hours. Constructs were subjected to dynamic compression (0-15%, 1 Hz) for up to 360 min with IL-1β and/or 10 μM PDTC (inhibits NFκB activation) [2]. Nuclear translocation of NFκB-p65 was analysed by immunofluorescence and confocal microscopy with a rabbit polyclonal antibody for the p65 subunit of NFκB (sc-372) and Hoechst 33258 to detect nuclei. The relative fluorescent intensity of cytoplasmic and nuclear NFκB-p65 was determined using the LCS software associated with the confocal microscope. For gene expression, RNA was isolated and reverse transcribed using oligo(dT) primers and the Stratascript™ RT [1]. Real-time qPCR assays coupled with molecular beacons were performed with cDNA, Brilliant® qRT-PCR Master Mix and analysed on the Mx3000P qPCR instrument. Relative quantification of IκB-α, iNOS, IL-1β and IL-4 signals were accomplished by normalizing each target to GAPDH and to the calibrator sample, by a comparative cycle threshold approach with PCR efficiencies incorporated into the analysis. Relevant parametric (ANOVA/post-hoc Bonferroni) tests were used to examine differences following log transformation of ratio values.

RESULTS:
Figure 1 illustrates the effect of IL-1β and dynamic compression on the nuclear translocation of NFκB at 60 min.

Fig. 1 Effect of IL-1β and dynamic compression (15%, 1 Hz) on NFκB activation in the nucleus and cytoplasm of chondrocytes cultured in unstrained and strained constructs with 0 or 10 ng.ml^-1 IL-1β and/or 10 μM PDTC for 60 min. 100 cells were visualized with FITC labelled antibody for the p65 subunit of NFκB (green) and Hoechst (blue).

In the absence of IL-1β, the location and distribution of NFκB-p65 was largely in the cytoplasm and appears to increase in concentration in the nucleus in strained cells (Fig. 1). NFκB-p65 appeared in the nucleus in unstrained cells cultured with IL-1β (Fig. 1). Stimulation by dynamic compression or PDTC did not completely inhibit nuclear translocation since a portion of NFκB-p65 remained in both the nucleus and cytoplasm. Co-stimulation with dynamic compression and PDTC indicated a significant proportion of NFκB-p65 in the cytoplasm. Furthermore, the application of dynamic compression increased cytoplasmic fluorescence intensity with IL-1β and / or PDTC illustrating that NFκB-p65 had occupied the cytoplasm (data not shown).

Figure 2 illustrates the temporal profile of IκB-α, iNOS, IL-1β and IL-4 expression in constructs subjected to dynamic compression with IL-1β and / or PDTC for up to 360 min. IL-1β increased IκB-α expression at 60 min (p<0.001; Fig. 2A) and either induced iNOS (p<0.001; Fig. 2B) and IL-1β (p<0.01; Fig. 2C) or inhibited IL-4 (p<0.05; Fig. 2D) expression at 360 min. These time-dependent events were partially inhibited by dynamic compression or PDTC with IL-1β. Co-stimulation by dynamic compression and PDTC favoured suppression of IκB-α, iNOS, IL-1β. In contrast, IL-4 expression was induced by dynamic compression in the presence and absence of IL-1β and/or PDTC.

Fig. 2 Temporal profile of dynamic compression (15%, 1Hz) on IκB-α (A), iNOS (B), IL-1β (C) and IL-4 expression (D) in unstrained and strained constructs cultured with 0 (▴) or 10 ng.ml^-1 IL-1β (☐) and/or 10 μM PDTC (□) for 60, 120 and 360 min (n = 8; ± SEM).

DISCUSSION:
In summary, this study explored the potential of IL-1β and dynamic compression to influence NFκB activation and expression of IκB-α in chondrocyte / agarose constructs. IL-1β increased NFκB-p65 nuclear activation and reached a threshold intensity causing transcription of its inhibitory protein, IκBα. Once expressed, IκB-α shuttles active NFκB-p65 back into the cytoplasm to switch off transcription. However, continuous activation with IL-1β enabled changes in NFκB nuclear and cytoplasmic activity and led to the induction and inhibition of the inflammatory (IL-1β), inducible (iNOS) and anti-inflammatory (IL-4) genes at later time points. These time-dependent events could be inhibited by co-stimulation with dynamic compression and the NFκB inhibitor, PDTC, suggesting that mechanical loading may impair IκB-α degradation and terminate transcription. An improved understanding of the interplay between biomechanics and cell signalling will provide key parameters for the safe application of pharmacological therapies, in conjunction with biophysical treatments for OA.

REFERENCES:

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