EFFECTS OF COMPRESSION AND TNF ON HUMAN CARTILAGINOUS ENDPLATE CELLS IN 3D AGAROSE CULTURE

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Introduction

Intervertebral disc (IVD) degeneration is the main cause of low back cases in young adults. The cartilaginous endplates (CEP) cover the cranial and caudal surfaces of the IVD and act to transmit compressive loads and transport water, nutrients, and waste in and out of the IVD [1]. Early CEP degeneration is likely to play a key role in IVD degeneration, but little is known about CEP mechanobiology and its changes in degeneration [1]. Investigating these changes is essential to elucidate how the CEP contributes to IVD pathology. We hypothesized that CEP cells would behave similarly to articular chondrocytes. Hence, dynamic compression would be sufficient to induce anabolism, while stimulation with pro-inflammatory cytokines would induce catabolism.

Methods

Human CEP cells were expanded until passage 3 or 4, then seeded at a density of 7.5x106 cells/ml into 2% agarose carriers (dimensions: 6 mm ⌀, 3 mm height) and cultured 5 days for phenotype recovery. Cell-agarose carriers were placed in custom-made chambers, stimulated with 10 ng/ml TNF, and dynamically compressed to ~7% strain for one hour at 1.5 Hz daily for up to 14 days. Carriers were collected on Days 0, 7, and 14 for downstream analysis. For statistical analysis, nonparametric distribution was assumed and a Kruskal–Wallis test then Dunn's multiple comparisons test done; $p < 0.05$ was considered statistically significant. A previously developed articular cartilage chondrocyte (ACC) mechanotransduction regulatory network model (RNM) was used to predict protein activation levels by initial mechanoreceptor perturbations representing dynamic compression (α5β1, αvβ3), physioosmotic pressure (TRPV4), tensile strain (αvβ5), and TNF. [2]

Results

After 14 days, TNF-stimulated cell-agarose carriers showed a trend ($p = 0.1$) towards decreased expression of anabolic gene aggrecan (ACAN). Specifically, dynamically loaded TNF-stimulated carriers showed significantly less ACAN expression ($p=0.0436$) than dynamically loaded control carriers after 14 days, but not after 7 days (Fig 1a). While there was no change in expression of collagen II (COLII), expression of collagen I (COLI) trended towards lower expression in TNF-stimulated carriers after 7 and 14 days ($p = 0.0663$ and $p = 0.0549$, respectively). Catabolic genes matrix-metalloproteinase 3 (MMP3) and interleukin 6 (IL-6) showed a trend towards increased expression in TNF-stimulated dynamic carriers when compared to the controls ($p = 0.2169$ and $p = 0.1240$, respectively) (Fig 1b, 1c). Predicted protein activations by the RNM matched experimental results for ACAN, but showed opposing results for MMP3 and IL6 under dynamic compression and TNF-stimulation (Fig 1d).

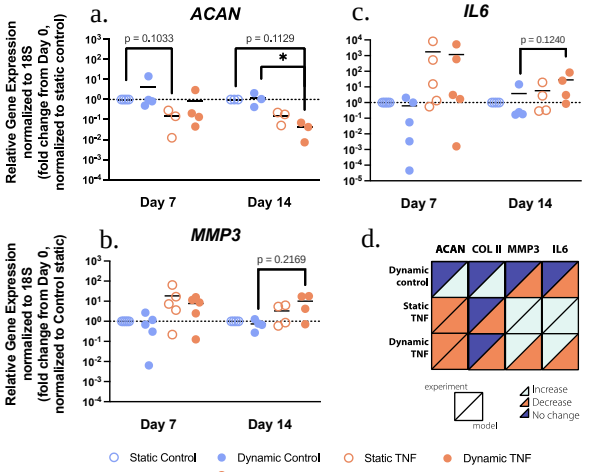

Figure 1: Relative gene expression of CEP cells after 7 and 14 days of 7% mechanical loading: a. ACAN, b. MMP3, and c. IL6. Results were normalized to Day 0 and the static control condition. (n = 3-5) d. Comparison between experimental gene results and predicted protein activation from the RNM

Discussion

This study demonstrated that TNF was sufficient to induce a catabolic response in human CEP cells through the downregulation of ACAN and the upregulation of MMP3 and IL6. Interestingly, TNF appears to have a greater effect on ACAN and COL1 than COL2 within the CEP. Further, response to TNF appeared enhanced by dynamic compression. Significant changes did not happen until after 14 days of culture, revealing a time dependent response to TNF-stimulation and dynamic compression. Differing responses between experiment and NM suggest that the CEP has a cellular response distinct from that of ACC. However, gene expression data might not match protein quantity and 7.5% strain or the scaffold material may not lead to full activation of α5β1 and αvβ3 and downregulation of pro-inflammatory regulation pathways, as assumed by the ACC regulatory network. Further, the model does not consider paracrine communication effects. Future work will include proteomic analysis by mass spectrometry.

References

1. K. Crump et al (2023) JOR Spine 6(4):e1294.
2. M. Segarra-Queralt et al (2023) Front Bioeng. Biotechnol. 3;11:1006066

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