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In-Vitro and In-Silico Investigation of Dynamic Compression on Cartilage Endplate Cells in Agarose

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INTRODUCTION: The cartilage endplate (CEP) covers the top and bottom of the intervertebral disc (IVD) and acts to transmit compressive loads and transport water, nutrients, and waste in and out of the disc. Early cartilage endplate (CEP) degeneration is likely to play a key role in IVD degeneration, but little is known about CEP mechanobiology and its changes in degeneration. Investigating these changes is essential to elucidate how the CEP contributes to IVD pathology.
METHODS: *In-vitro*: Bovine-tail CEP cells were expanded until passage three. Afterwards, a 1:1 mixture of CEP cells and agarose was pipetted into silicon molds to create 2% agarose and 1x107 cells/ml carriers, 6 mm diameter and 3 mm thickness, and cultured two days for phenotype recovery. Cell-agarose carriers were placed in custom-made chambers, stimulated with 10 ng/ml TGF-β1 throughout the entirety of the experiment and dynamically compressed up to 7% strain for one hour at 1.5 Hz every day for up to 14 days. Those not dynamically loaded experienced the constant weight of the chamber lid exerting ~5.1 Pa per carrier. Carriers were collected on Days 0, 7, and 14 for downstream analysis of cell viability, gene expression, and glycosaminoglycan (GAG) content.
In-silico: A 2D axisymmetric porohyperelastic, compressible, Neo-Hookean finite element model (FEM) of a cell-agarose carrier was developed in Abaqus using literature-derived material properties and loaded with dynamic compression as in the in-vitro experiment. A previously developed mechanotransduction network model was used to predict protein activation levels by initial mechanoreceptor perturbations standing for dynamic compression (α5β1, αvβ3), physioosmotic pressure (TRPV4), tensile strain (αvβ5), plus chondrogenic media (TGF-β). Predicted protein activation was normalized by baseline conditions.
RESULTS: After seven and 14 days of culture, cell-agarose carriers in all conditions demonstrated significantly increased expression of anabolic genes aggrecan (*ACAN*) 2-200x, collagen II (*COL II*) 3-1000x, and GAG/DNA content 2.5-5x, alongside decreased expression of catabolic gene matrix metalloproteinase 3 (*MMP3*). Reaction forces from FEM (0.07N) matched force data collected during loading (0.06N). The FEM showed that hydrostatic pressure varies from center to edge of carrier. General trends of increased/decreased gene expression and protein activation matched between experimental and network model results.
DISCUSSION & CONCLUSIONS: A novel framework coupling 3D cell culture with *in-silico* methods is presented, in which the FEM provides details about loads experienced at each point within the carrier, while the network model uses these mechanical cues and environmental perturbations to predict protein expression and identify key proteins for future analysis.

Keywords: Intervertebral disc / spine and their disorders, Biomechanics / biophysical stimuli and mechanotransduction