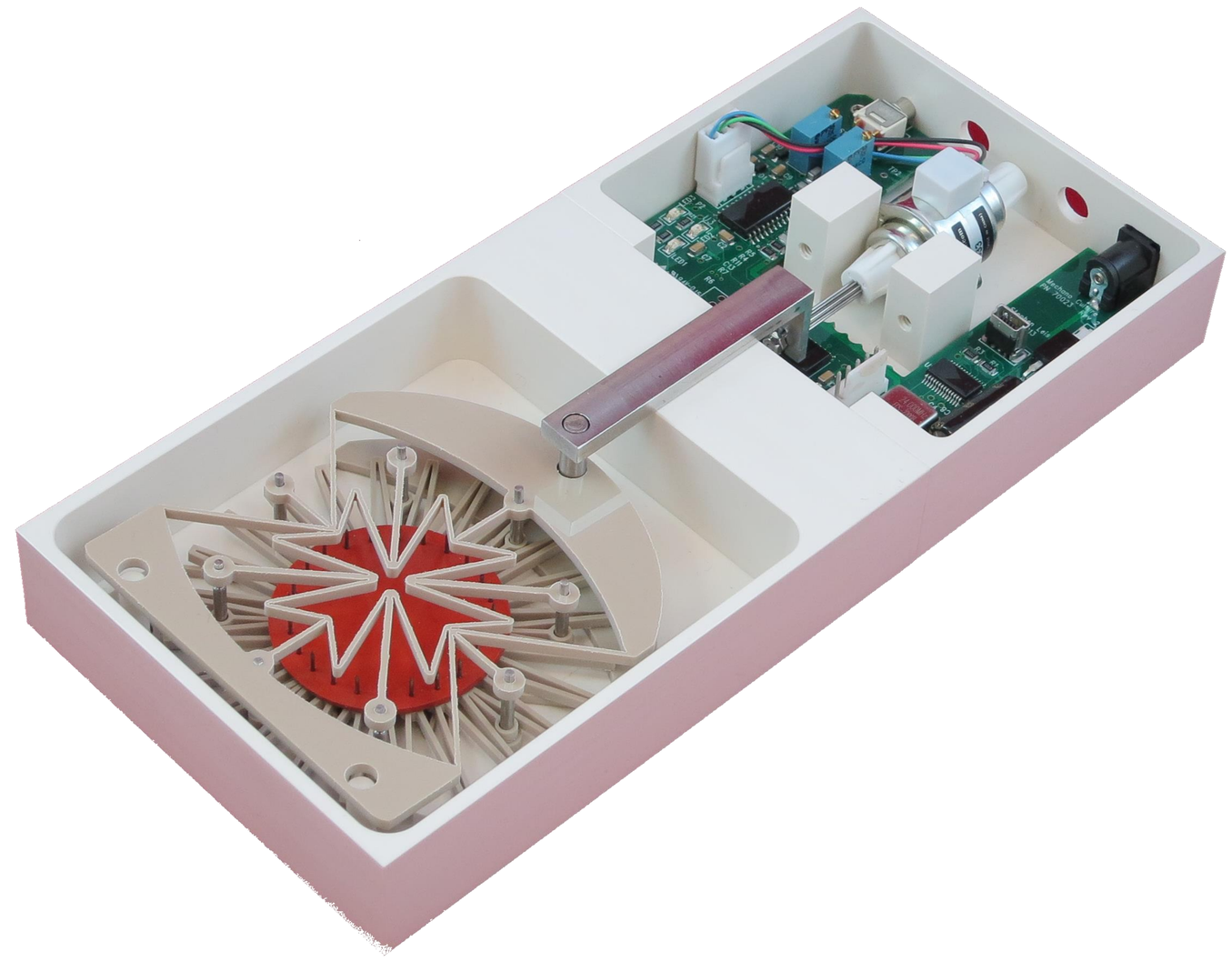
Mechanical Stimulation of Intervertebral Disc Cells

Overview

The MechanoCulture B1 is used to provide multi-axial stretch stimulation to cell cultures. In this study, mouse intervertebral disc (IVD) cells were stimulated to study changes in gene expression.



Introduction

The intervertebral disc (IVD) is a complex structure essential for spine stabilization, load bearing, and movement. It is composed of three distinct yet interdependent tissues: the central nucleus pulposus (NP), the outer annulus fibrosus (AF), and the cartilage endplates that anchor to the vertebrae. Although physiological loading is essential to disc homeostasis and stimulates increased extracellular matrix synthesis, excessive loading induces a cascade of non-reversible cell-mediated responses leading to disruption of tissue structure and function. Differences between the effects of mechanical loading on the distinct cell types of the IVD are poorly understood, as are the cellular pathways that regulate these responses. The culture of primary mouse IVD cells using high-density micromass cell cultures can permit the maintenance of the AF phenotype. This cell culture strategy can be used to access changes in AF cell gene expression following exposure of cells to cyclic multi-axial strain using the MechanoCulture device.

Preparation

To set-up the MechanoCulture device, a 0.005” thick silicone membrane was mounted to the 24 pins of the MechanoCulture carrier. All cell-contacting components were then sterilized using a standard autoclave protocol. Additional un-mounted membranes used as non-stretched controls. After sterilization and assembly, 2mL of pre-treatment media (DMEM-F12 containing 50%FBS, 10% P/S) was pipetted onto the membrane and the assembly was then placed in a tissue culture incubator overnight. The was done to enhance cell attachment to the membrane.

After aspirating the pretreatment media, 400,000 cells were seeded onto the membracein a micromass in a volume of 1.2mL of culture media (DMEM-F12+10%FBS). After overnight incubation, the membrane and carrier assembly was transferred using sterile forceps to the MechanoCulture test chamber. The chamber was flooded using culture media, first filling the chamber beneath the membrane. Media was then carefully added on top of the membrane so as not to shear cells off of the surface.

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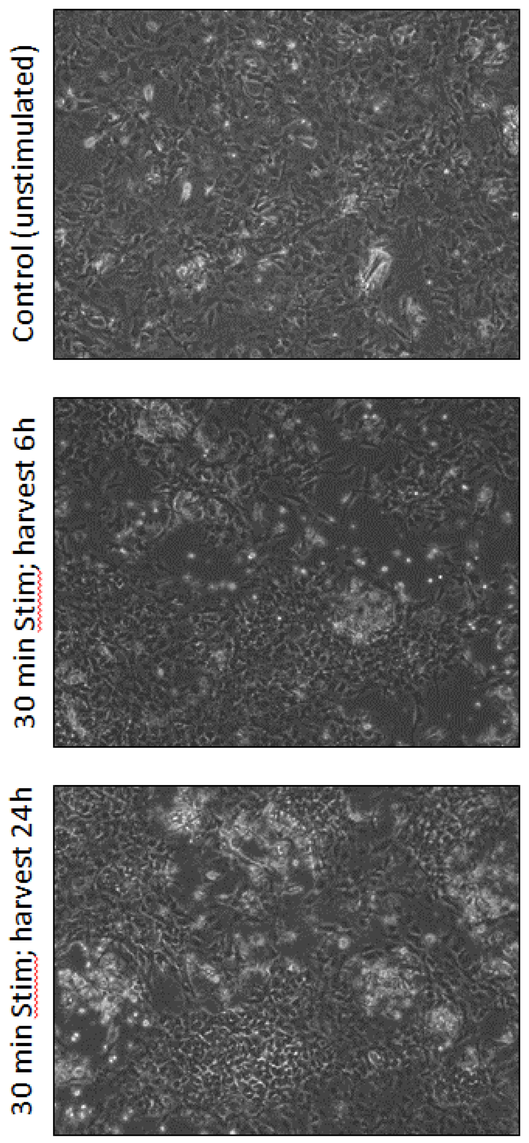
Protocol

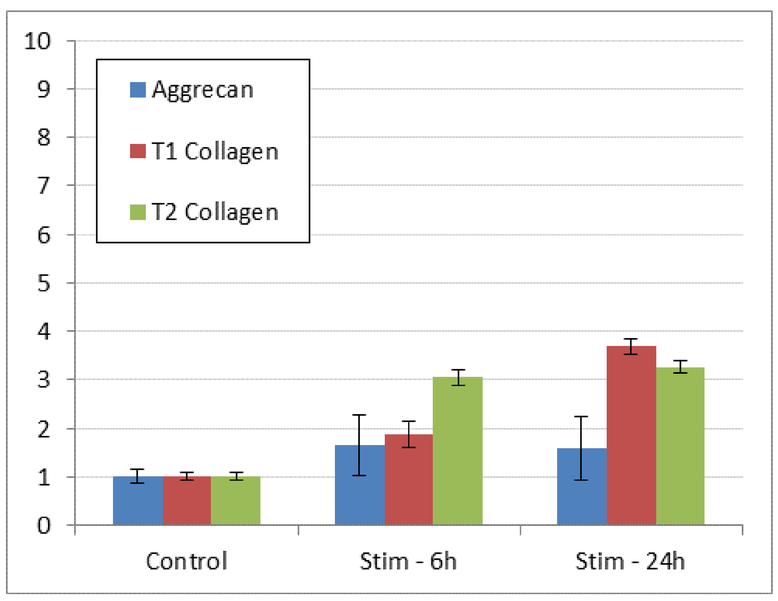
Twenty four hours following the initial plating, cells were subjected to mechanical loading. The protocol was to biaxially stretch the membrane by 10% strain at 1 Hz for 30 minutes. As a control case, sterilized membranes used for controls were transferred using sterile forceps to 6 cm cell culture dishes and were pretreated and plated with cells as described above. These control plates enabled user to monitor cell growth, viability and morphology of cells exposed to the silicone membrane.

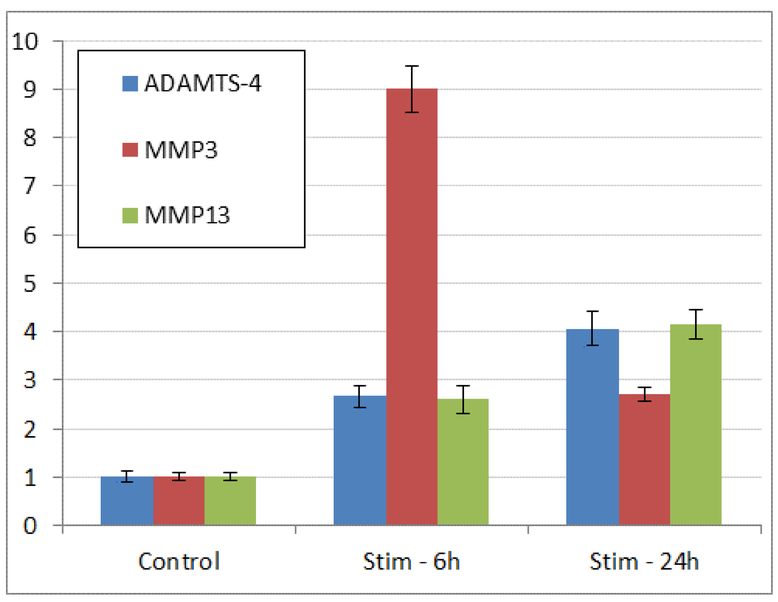
Cultures were harvested at 6 and 24 hours following stimulation for RNA extraction and gene expression analysis. After stretch sequence the stretching assembly was and disassembled. The membrane was carefully removed from mounting pins and placed into 6cm dish. Morphology of live cells was imaged immediately using phase/contrast. To harvest cells, 1mL of PBS was added directly on top of the membrane and cells were detached using a cell scraper. The cells in PBS were centrifuged to obtain a cell pellet. The cell pellet was subsequently suspended in 1mL of Trizol for subsequent RNA extraction and gene expression analysis.

Conclusions

Cell morphology and gene expression were assessed at 6 and 24 hrs following a single 30 min exposure of AF cells to biaxial stretch. Preliminary experiments suggest changes in cell morphology in stretched cells compared to non-loaded controls, producing cells with a more elongated, spindle-like morphology. SYBR-based Real time PCR assessment of gene expression suggests increased expression of genes encoding both extracellular matrix genes as well as matrix degrading enzymes. These preliminary findings are in keeping with previous studies which demonstrated that exposure of AF cells to cyclic tensile strain induces frequency-depending changes in gene expression including the induction of catabolic gene expression (Hamish et al, 2010).







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