Human Disc-on-a-Chip: Microfluidic and Hydrogel Systems to Model Neuroinflammation Underlying Intervertebral Disc Degeneration

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INTRODUCTION: Discogenic back pain is primarily caused by intervertebral disc (IVD) degeneration, which is driven by an imbalance of extracellular matrix (ECM) homeostasis, an increase in inflammation, and nociception. Animal and two-dimensional *in vitro* models have provided valuable insights but are lacking in recapitulating the human IVD phenotype within a physiologically relevant three-dimensional (3D) niche. Herein, we aimed to develop an IVD model integrating a microfluidic gradient device with ECM-based hydrogels, mimicking the heterogeneous IVD. We hypothesized that a human disc-on-a-chip model, utilizing a microfluidic and hydrogel system, mimics the heterogeneous 3D microenvironment of the disc that maintains the phenotypic markers of nucleus pulposus (NP), annulus fibrosus (AF), and sensory neurons.

METHODS: Disc-on-a-chip was fabricated using stereolithography with PDMS. incorporating variations in microfluidic channel and chamber geometries, with various hydrogel concentrations and compositions, to mimic the NP, AF, and sensory regions integrated with a microelectrode array. Computational simulations were performed to analyze flow rate and velocity within the microfluidic system. The microfluidic slab was assessed for surface hydrophilicity and roughness. Hydrogels were characterized for their mechanical properties. The inner core hydrogel consisted of hyaluronic acid (HA) and Collagen type II (COLII) (HA/COLII) for NP, surrounded by COLI hydrogel for AF, with the sensory region positioned peripherally. Human NP and AF cells, as well as iPSC-derived sensory neurons, were incorporated within hydrogels and assessed for viability, metabolic activity, phenotypic marker expression, and local field potentials. An inflammatory milieu disc-on-a-chip will be simulated using degenerative human NP and AF cells by tuning gradient concentrations of neurogenic mediators (IL-1β and NGF). The model will be assessed for ECM catabolic activity, inflammatory markers (IL-6, TNF), pro-nociceptive markers ($NaV_{1.7}$, TRPV1, TrkA), neurite length (peripherin, PGP9.5), and local field potentials.

RESULTS: Computational simulations demonstrated that increasing pressure led to higher shear stress and velocity, whereas curved-microchannel designs resulted in uniform flow and reduced pressure. A 60-second treatment time with oxygen plasma resulted in optimal hydrophilicity and surface roughness. HA/COLII hydrogel at 3 mg/mL exhibited greater stiffness, viscosity, and shear stress, while 2 mg/mL of hydrogel provided optimal viscosity with lower resistance. Morphologically, NP cells demonstrated a rounded shape and were evenly distributed throughout the hydrogel. They exhibited higher expression of COL2A1, KRT19, ACAN, and Brachyury, AF cells expressed COL1A1 and FN, indicating the maintenance of region-specific phenotypes. We observed the expression of peripherin and PGP9.5, indicating the presence of sensory neurites in iPSC-derived sensory neurons.

DISCUSSIONS: The use of ECM-based hydrogels and microfluidic gradients facilitated the establishment of spatially defined NP, AF, and sensory neurons, hence informing region-specific phenotypes. Computational simulations showed the impact of microchannel design on shear stress and flow homogeneity, whereas hydrogel optimization preserved cell viability and tissue-specific phenotype. The expression of peripherin and PGP9.5 validated the integration of sensory neurons.

CONCLUSION: Human disc-on-a-chip mimics the heterogeneous structure and mechanical property of the IVD, which provides a conducive 3D microenvironment for AF and NP cells to maintain their cell phenotype. This platform provides a physiologically relevant 3D model to investigate inflammation and nociception underlying IVD degeneration, enabling a rapid disease prototyping for therapeutic testing.

SIGNIFICANCE: This approach provides a human-relevant substitute for animal and 2D models, facilitating mechanistic investigations of inflammation and nociceptive signaling in IVD degeneration. It provides a disease model for evaluating anti-inflammatory and anti-nociceptive treatments for discogenic back pain.

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