A Microfluidic 3D Disc-neuron Platform for Investigating Discogenic Pain and Therapeutic Modulation

Yuan Xing[1], Li Xiao[1], Li Jin[1], Xudong Li [1,2]
Departments of [1]Orthopaedic Surgery, [2]Biomedical Engineering, University of Virginia, Charlottesville, VA, USA

Presenting author email: yx6b@virginia.edu
Disclosures: We have nothing to disclose.

INTRODUCTION: Intervertebral disc (IVD) degeneration is a major contributor to chronic low back pain, characterized by extracellular matrix breakdown, inflammation, and aberrant sensory nerve ingrowth. Existing in vitro models often fail to recapitulate the disc's complex cellular architecture and degenerative microenvironment. To address this, we developed a biomimetic 3D disc, featuring a central nucleus pulposus (NP)-like core surrounded by a centric aligned annulus fibrosus (AF). Incorporation with a microfluidic innervation-on-chip system, we investigated how disc degeneration influences sensory neuron activation and innervation.

METHODS: AF and NP cells were isolated from healthy lumbar intervertebral discs of C57BL6 mice (Jackson Laboratory) with the Institutional Animal Care and Use Committee (IACUC) approval. AF cells were encapsulated in a fibrin–collagen gel (10 mg/mL fibrinogen,1 mg/mL collagen) and crosslinked with thrombin. Over 48 hours, gel contraction induced circumferential alignment of AF cells. A central cavity (3mm) was created and filled with NP cells embedded in a softer fibrin gel (5 mg/mL fibrinogen), reconstructing native AF–NP anatomy. Discs were cultured in DMEM-F12 with 10% FBS. Degeneration was induced by using IL-1β (10 ng/mL, 24 h at 37 °C). For neuronal studies, lumbar dorsal root ganglia (DRG) were carefully dissected, enzymatically digested, and seeded onto poly-lysine-coated microfluidic biochips. Neurons were allowed to establish for 24 hours before introducing discs (with or without IL-1β treatment), in the disc chamber. Neurite extension and disc-neuron interactions were monitored over 3-day days via innervation channels. Neurite outgrowth was quantified using ImageJ software following β-III-tubulin immunostaining. DRG neuron activation was assessed by dynamic calcium imaging using Fluo-4. Data were expressed as mean ± SEM and analyzed using one-way ANOVA or t-test, with statistical significance defined as a p-value < 0.05.

RESULTS SECTION: The engineered 3D discs captured key disc architecture: AF cells aligned circumferentially around the periphery, while NP cells oriented randomly within the central jelly matrix (Fig. 1b,c). Cell orientation analysis confirmed anisotropic alignment of AF cells and isotropic distribution of NP cells, mimicking native tissue. IL-1β stimulation induced typical degenerative changes, including downregulation of anabolic genes (ACAN, COL2) and upregulation of inflammatory and catabolic genes (IL-1, IL-6, MMP13), along with elevated VEGF expression (Fig. 1e). In on-chip culture, degenerative 3D discs significantly enhanced neurite outgrowth across microfluidic channels compared to intact 3D discs or disc free controls (Fig. 2a). Quantitation analysis revealed increased neurite count and directional length in degenerative disc conditions, which were attenuated by curcumin treatment (Fig. 2b,c). Calcium imaging showed that neurons exposed to degenerated discs exhibited frequent, high-amplitude calcium transients (Fig. 3c), indicating hyperexcitability, whereas neurons cultured with non-degenerated discs maintain low baseline activity (Fig. 3a-c).

DISCUSSION: This study presents a physiologically relevant in vitro disc–neuron interface that captures key structural and inflammatory features of disc degeneration. The 3D disc model replicates AF and NP organization and responds to inflammatory stimuli. Integration with a microfluidic innervation-on-chip platform revealed that degenerative discs promote neurite ingrowth and neuronal hyperexcitability, both hallmarks of painful disc degeneration. The suppression of neurite that degenerative discs promote sensory neurite ingrowth and neuronal hyperactivity. The attenuation of neurite extension by curcumin indicates the model's potential for preclinical drug testing.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings demonstrate that disc degeneration attracts sensory nerve ingrowth and activation, offering mechanistic insight into discogenic pain. The combined 3D discs and innervation-on-chip model provides a physiologically relevant, controllable platform for studying disc–nerve interactions, identifying therapeutic targets, and evaluating candidate interventions. By bridging tissue engineering and neurobiology, this model offers a clinically relevant tool for developing treatments aimed at alleviating chronic discogenic pain.

ACKNOWLEDGEMENTS: We are grateful for financial support from NIH NIAMS R01AR078888 andR21AR078547.

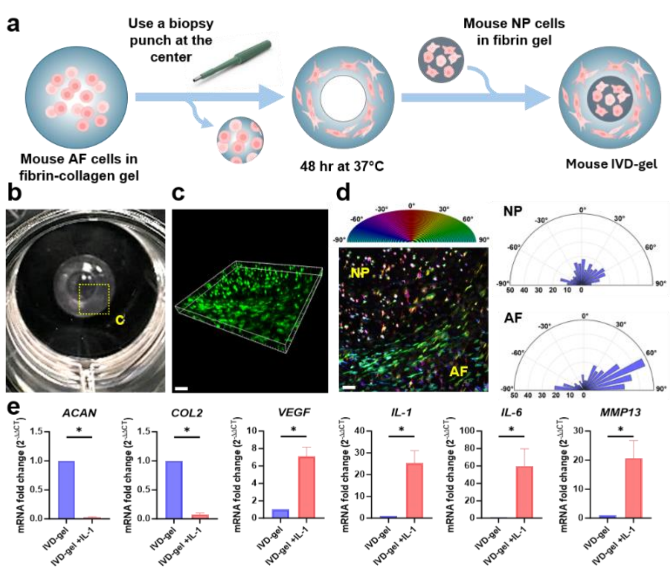

Figure 1. Construction and characterization of an intervertebral disc (IVD)-mimicking hydrogel model (IVD-gel). The IVD-gel was fabricated by embedding AF cells in a fibrin–collagen hydrogel that contracted to form an aligned outer ring, followed by creating a central cavity with a biopsy punch and filling it with NP cell–laden gel to reconstruct disc-like architecture (**a, b, c**). Cell orientation analysis confirmed anisotropic alignment of AF cells and isotropic distribution of NP cells (**d**). The IVD-gel was treated with IL-1β (10 ng/mL, 24 h at 37 °C) to induce degeneration (**e**). Scale = 100 µm. *p<0.05 by t-test, n=4; error bar =SEM.

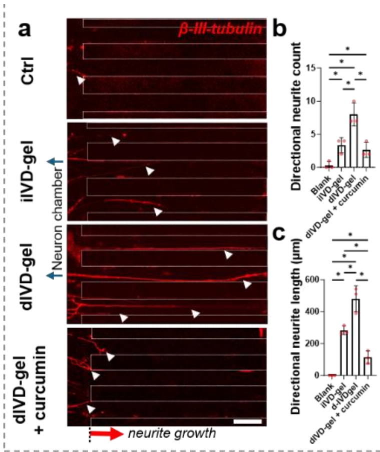

Figure 2. Degenerative disc induced nerve innervation on-chip. Mouse DRG neurons and IVD-gel (pretreated with or without IL-1β) were co-cultured for 3 days on a microfluidic innervation-on-chip platform we designed. Directional neurite extension (arrowheads, β-III tubulin–stained) through the channels **(a)** was quantified, with lengths ranked in descending order as follows: degenerative IVD-gel (dIVD-gel) > intact IVD-gel (iIVD-gel) > dIVD-gel + curcumin (5 µg/mL) > control (no disc). (**b, c**). Scale = 100 µm. *p<0.05 by one-way Anova, n=5; error bar =SEM.

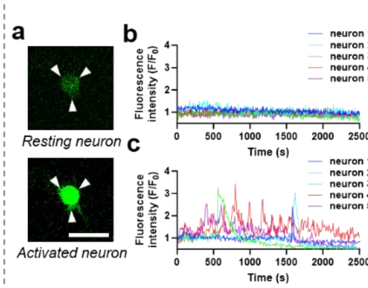

Figure 3. Ca^{2+} traces of DRG neurons cocultured with degenerative IVD-gel on-chip. DRG neurons were activated during the coculture with degenerative IVD-gel (**a**) and monitored by Fluo-4. The F/F_0 traces revealed more frequent and higher-amplitude calcium transients in neurons cocultured with dIVD-gel (**c**) compared to those with iIVD-gel (**b**). Scale = 50 µm.