Hyaluronan-Coated Aligned Collagen Hydrogel Stabilizes Intervertebral Discs *via* Fibrotic Remodeling of the Annulus Fibrosus

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INTRODUCTION: Chronic low back pain (LBP) is a major global health burden, with degeneration of the intervertebral disc (IVD) recognized as a key underlying factor. IVD is a mechano-physically active tissue that relies on the outer annulus fibrosus (AF) to absorb stresses while maintaining the spine functionality. When the AF is damaged, often due to recurrent axial loading, it can lead to disc rupture, extrusion of the nucleus pulposus (NP), and ultimately disc degeneration. Current treatment approaches to AF restoration are limited and do not completely restore disc function. To bridge this gap, we created a hyaluronan-coated type I collagen (Col-I HA) hydrogel scaffold that supports AF tissue healing while mechanically stabilizing the degenerative disc.

METHODS: We developed a hyaluronan-coated, aligned type I collagen (Col-I HA) hydrogel scaffold using electrocompaction method to mimic the aligned structure of an AF) (fig. 1A). Fibre alignment and molecular integrity were confirmed using scanning electron microscopy (SEM), attenuated total reflectance/Fourier transform electron microscopy (ATR/FTIR), and circular dichroism (CD) spectroscopy (fig. 1B), while mechanical testing demonstrated tensile strength comparable to native tissue. *In vitro* studies with primary AF cells and $CD90^{+}/CD73^{+}$ rat BMSCs showed significant cell viability and fate (fig. 2A), and extracellular matrix (ECM)-related genetic expressions (fig. 2B). Ex vivo rat IVD explant and the *in vivo* rat AF defect model (female rats were used due to growth and behavioral consistency) was developed and investigated to examine architectural integration, behavioral assessment, magnetic resonance imaging (MRI)-based recovery, histological remodeling, and biomechanical restoration (fig. 3A).

RESULTS: The fabricated Col-I HA hydrogel scaffolds demonstrated well aligned collagen fibrils with conserved triple-helix structure, as validated by ATR-FTIR, CD spectroscopy, FE-SEM, and FFT investigations (fig. 1B). Mechanical testing showed good tensile strength and Young's modulus under dry conditions, but reduced stiffness in wet environments due to the hydration properties of HA. *In vitro*, AF cells and $CD90^{+}/CD73^{+}$ RBMSCs demonstrated enhanced cellular alignment, proliferation, and expression of regenerative (Col-I, Acan, Mkx, Scx) and contractile/fibrotic markers (Acta2, α-SMA, CD146) (fig. 2B). Ex vivo and *in vivo* rat models demonstrated restoration of mechanical characteristics and significant ECM deposition at the site of annular defect. Histological and immunohistochemical investigations revealed increased collagen and GAG levels along the developed fibrous matrix. Upregulation of TGF-β1, FN, α-SMA, and CD146 indicates epithelial to mesenchymal transition (EMT)-driven myofibroblast activation, which contributes to fibrotic remodeling. Scx was observed to be highly expressed in AF and outer fibrotic regions, indicating its active participation in fibroblast-myofibroblast transition and cytoskeletal framework. Col-I HA restored biomechanical integrity, facilitated tissue remodeling, and encouraged reparative fibrosis in deteriorating IVDs (fig. C, D &E).

DISCUSSION: Our findings show that the developed Col-I HA scaffold mimics the AF structure, encourages aligned cell growth, and facilitates extracellular matrix repair. Furthermore *in vitro* and *in vivo* studies demonstrated improved mechanical characteristics, collagen and GAG deposition, and fibrotic stability at the site of implantation. Most significantly, the scaffold remained intact after implantation, fostering the development of newly deposited matrix and functional recovery. Despite fibrotic markers were elevated, they indicated to participate in a reparative role in tissue remodeling. Overall, this study shows a promising biomaterial-based technique for reinstating IVD biomechanics and structural stability, which might be useful as a potential therapeutic strategy for IVDD.

SIGNIFICANCE/CLINICAL RELEVANCE: The Col-I HA hydrogel scaffold is a biomimetic, and mechano-physically active for annulus fibrosus repair and functional stabilization of damaged intervertebral discs.

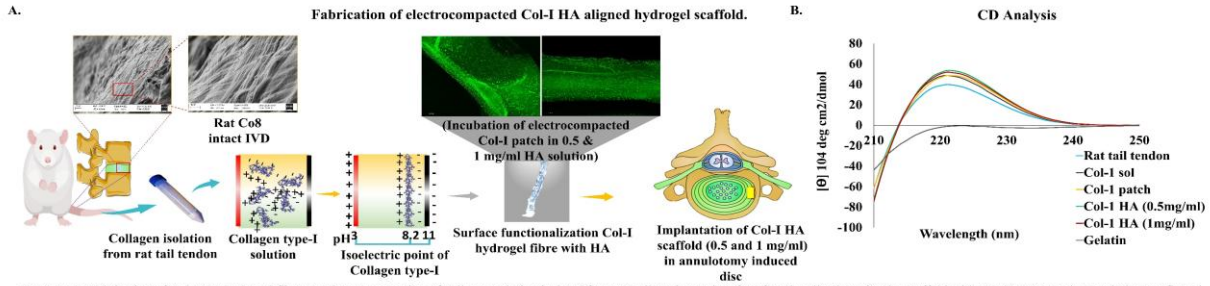

Figure 1 A: Fabrication of Col-I HA hydrogel fibre *via* electrocompaction of collagen solution isolated from rat tail tendon and surface functionalization of Col-I scaffold with HA (0.5 & 1 mg/mL) solution confirmed via visualized images from confocal microscopy. **(B).** CD analysis spectra of rat tail tendon, Col-I solution, Col-I scaffold, Col-I HA (0.5 mg/mL), Col-I HA (1 mg/mL), and gelatin.

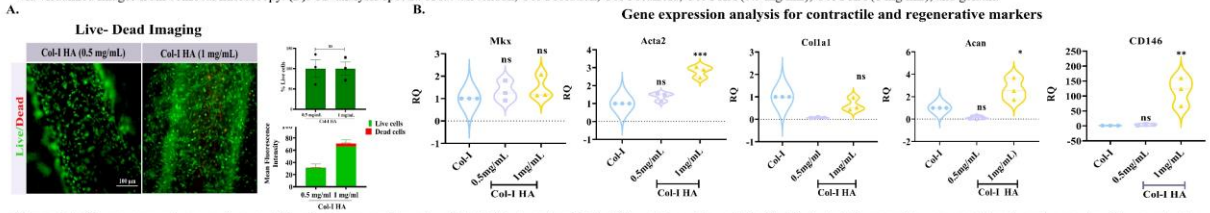

Figure 2 A: Fluorescence microscopy images of live dead assay performed on Col-I HA hydrogel scaffolds (0.5 mg/mL and 1 mg/mL). Graphical plots for mean fluorescence intensity and percentage live count cells **(B).** Relative gene expressions of cells assessed for Col1a1, Acan, Mkx, Acta2 and CD146 gene in AF cells.

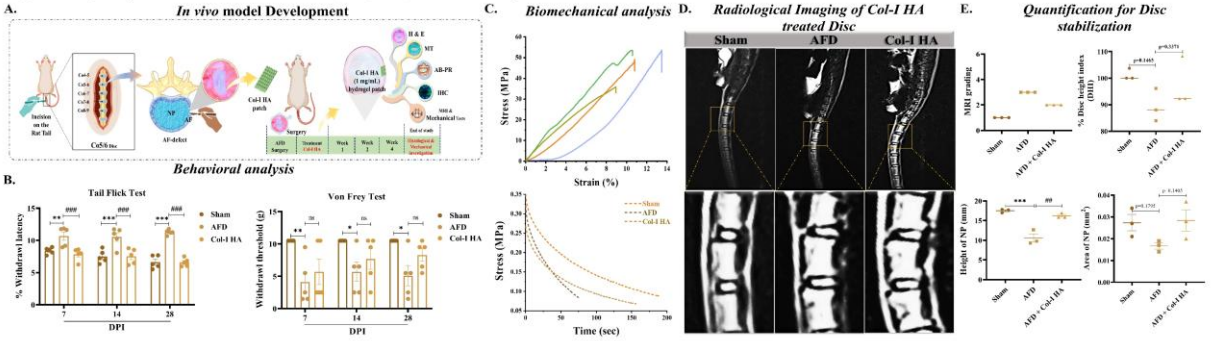

Figure 3 A: Development of *in vivo* rat tail AFD model. **(B).** Behavioural assessment for nociceptive threshold determination *via* tail flick (thermal) and von Frey filament test (mechanical allodynia). **(C). B**iomechanical investigations *via* stress *vs.* strain and stress relaxation of the discs treated with Col-I and Col-I HA (1 mg/mL). **(D&E).** *In vivo* analysis for structural and functional imaging of Col-I HA stabilized IVD *via* Magnetic resonance (MRI) imaging for MRI grade, disc height index, height and area of NP.