Investigating mechanosensitive alternative splicing in a 3D model of heart disease

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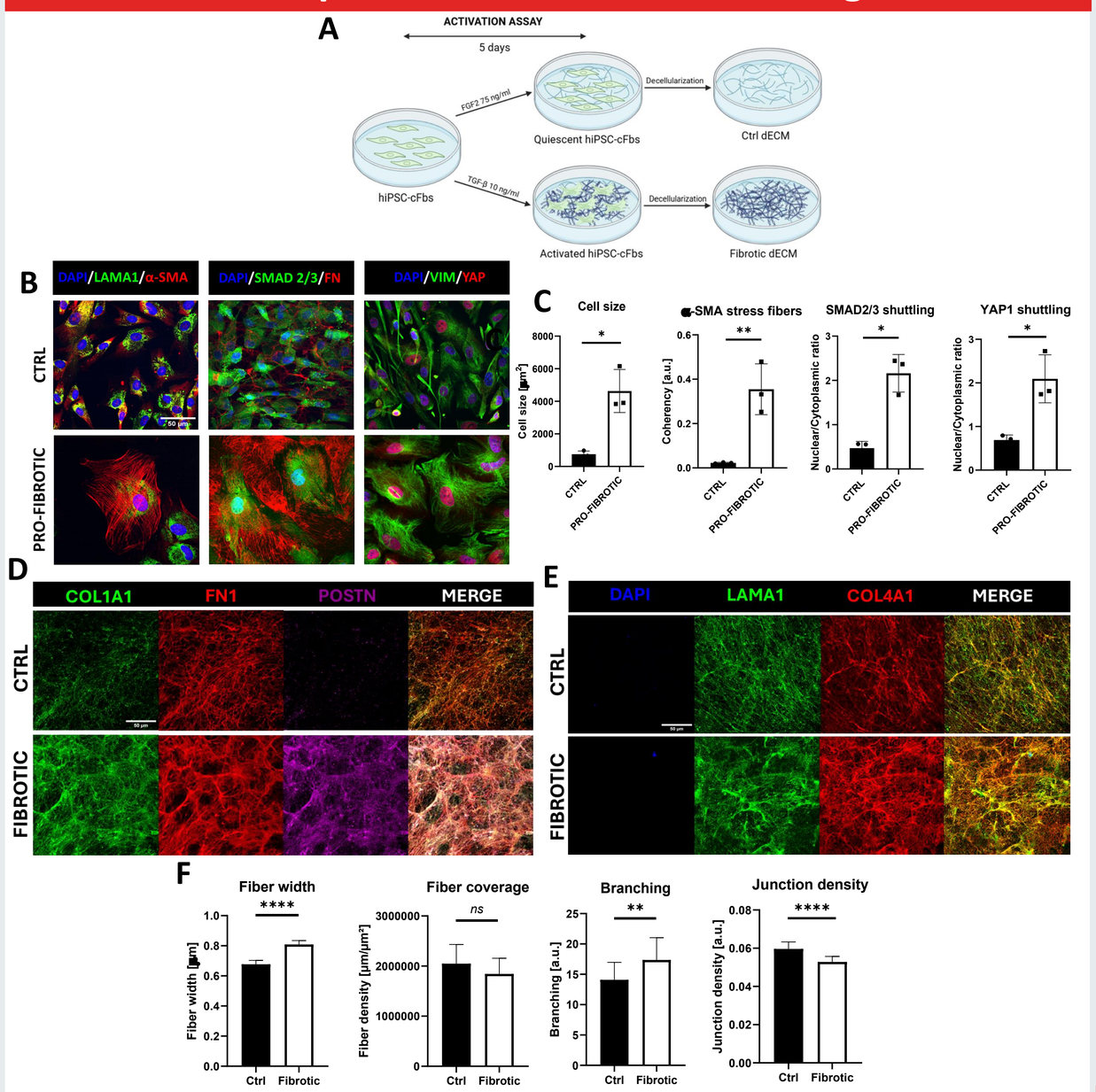
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Introduction

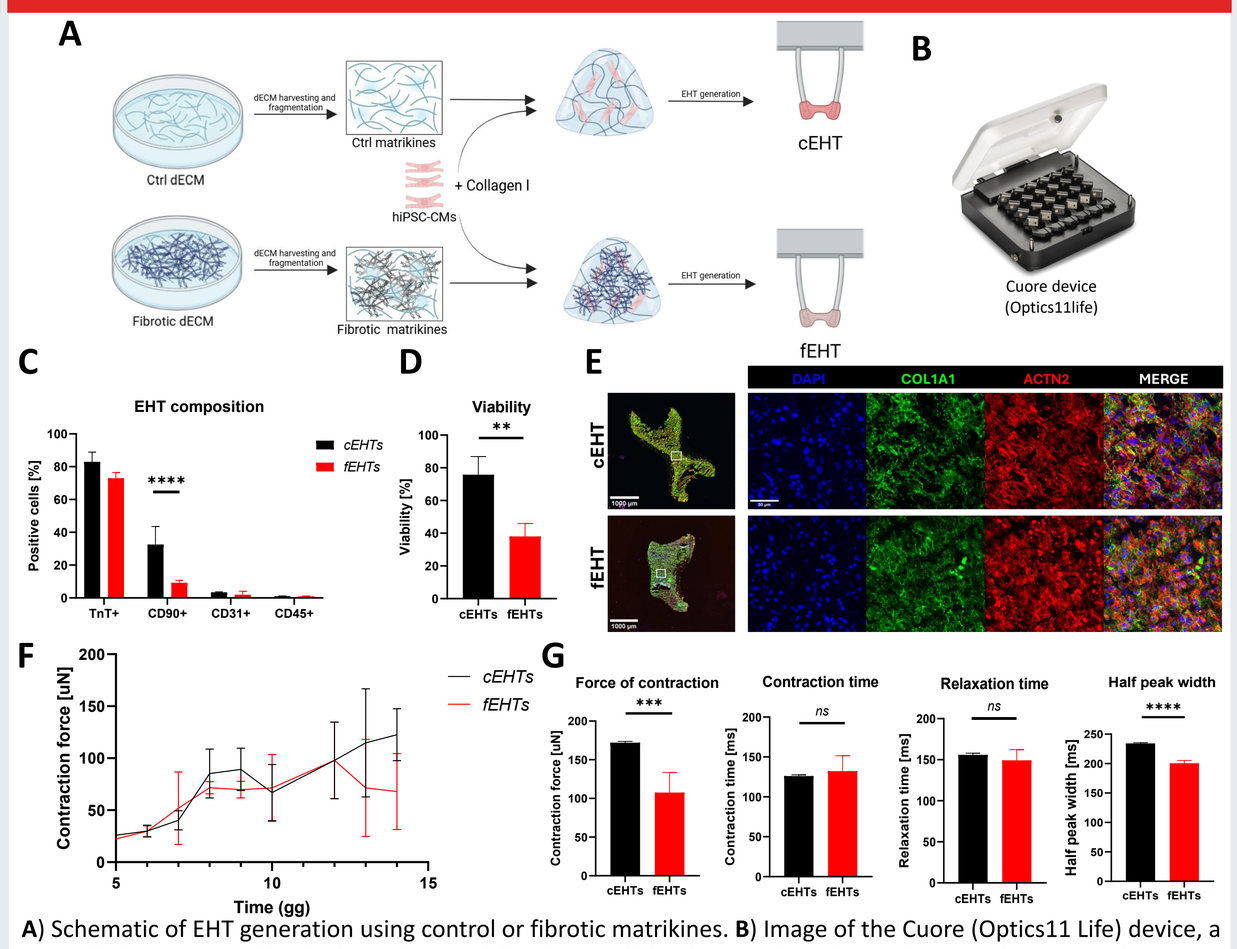
Cardiac fibrosis is a hallmark of heart failure, driving extracellular matrix (ECM) remodelling that alters myocardial architecture and disrupts cardiomyocyte function. Emerging evidence suggests that mechanical stress from this process rewires gene expression post-transcriptionally through alternative splicing (AS)[1], yet the underlying mechanisms remain poorly understood due to a lack of physiologically relevant human models. To address this, we developed a fully human, hiPSC-based 3D engineered heart tissue (EHT) platform to investigate the interplay between fibrotic mechanical cues and RNA metabolism. hiPSC-derived cardiac fibroblasts were activated with TGF-β1 to deposit a fibrotic ECM, which was then fragmented into soluble bioactive "matrikines". These were incorporated into EHTs with hiPSC-derived cardiomyocytes, and the resulting constructs were characterised using contractile function analysis, immunofluorescent imaging, flow cytometry and single-cell RNA sequencing (scRNA-seq).

TGF-β Drives Fibrotic Remodeling



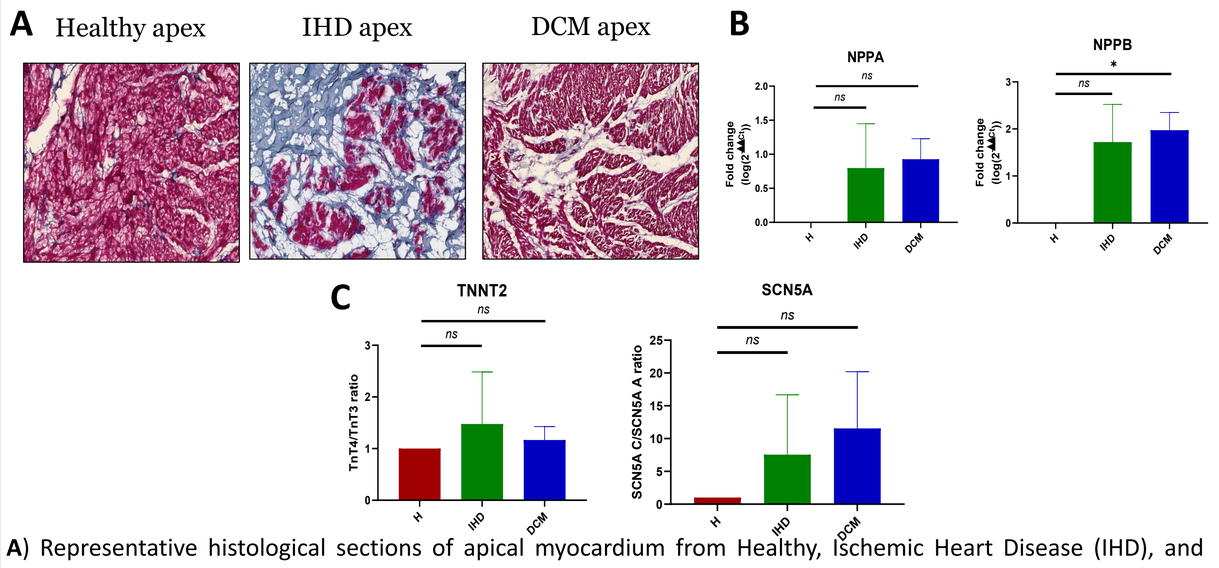
A) Schematic of hiPSC-cFb activation and dECM generation. **B**) Representative confocal images of control (CTRL) and pro-fibrotic (TGF-β1 treated) cells stained for key markers of myofibroblasts: laminin α1 (LAMA1, green) and α-smooth muscle actin (α-SMA, red); SMAD2/3 (green) and fibronectin (FN, red); vimentin (VIM, green) and YAP1 (red). **C**) Quantification of key myofibroblast features: cell size, α-SMA stress fibre coherency, and nuclear-to-cytoplasmic ratio of SMAD2/3 and YAP1. Representative confocal images of control and fibrotic dECM stained for key matrix components **D**) Collagen I (COL1A1, green), Fibronectin (FN1, red), Periostin (POSTN, magenta) and **E**) Laminin α1 (LAMA1, green) and Collagen IV (COL4A1, red). **F**) Quantification of ECM architectural features: fibre width, fibre coverage, branching points, and junction density.

Characterization of a 3D Cardiac Model of Fibrosis



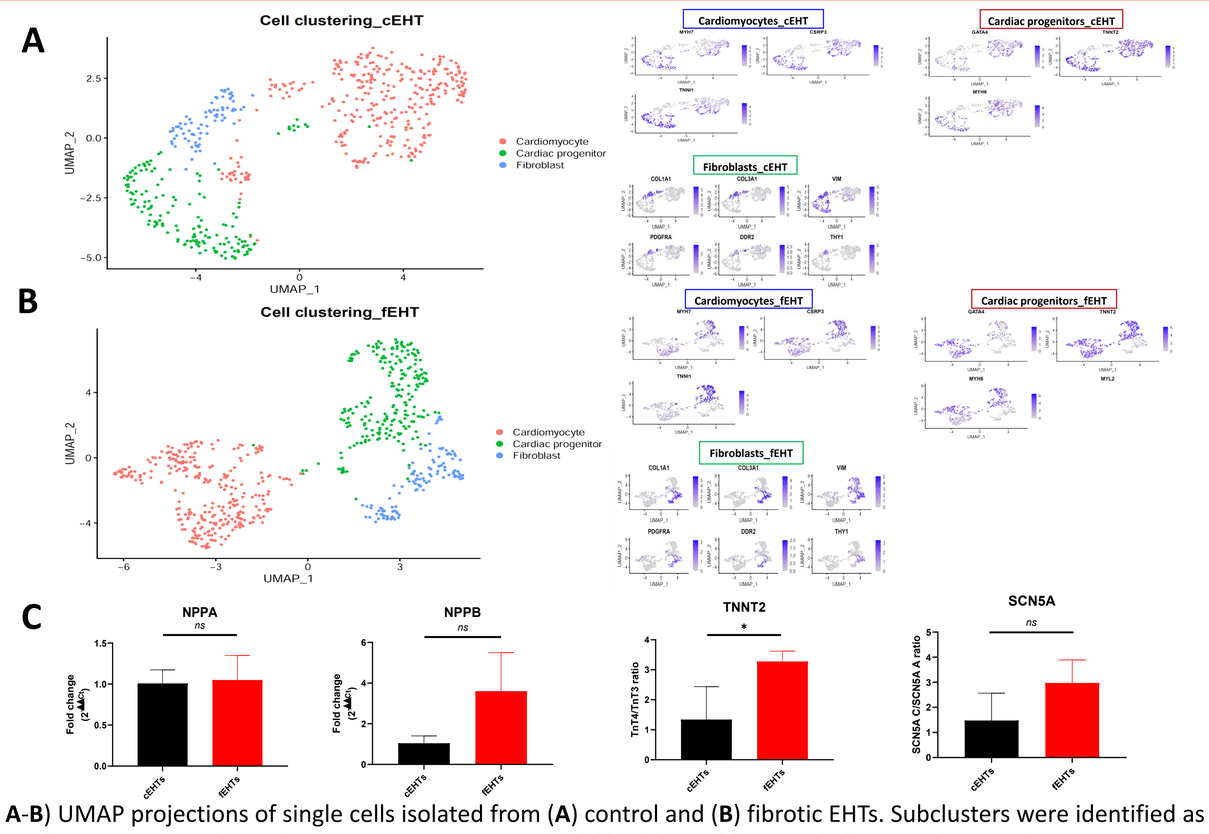
A) Schematic of EHT generation using control or fibrotic matrikines. **B**) Image of the Cuore (Optics11 Life) device, a 24-well plate-compatible platform designed for the parallel culture and real-time functional analysis of EHTs. **C**) Bar plot representing the percentage of cells positive for CD90, a fibroblast marker, TnT, a cardiomyocyte marker, CD45, an immune marker, and CD31, an endothelial marker, resulting from a flow cytometry analysis of cells dissociated from cEHTs and fEHTs. **D**) Quantification of viable cells dissociated from cEHTs and fEHTs by flow cytometry with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit. **E**) Representative immunofluorescence of EHTs stained for ACTN2 (cardiomyocytes, red), COL1A1 (matrix, green), and DAPI (nuclei, blue). **F**) Longitudinal monitoring of contraction force. **G**) Quantitative analysis of contractile parameters at a representative time point (day 14): contraction force, contraction time, relaxation time, half-peak width. Data are means ± SD. ***p<0.001; ****p<0.0001.

Pathological Splicing Profiles in Failing Myocardium



A) Representative histological sections of apical myocardium from Healthy, Ischemic Heart Disease (IHD), and Dilated Cardiomyopathy (DCM) donors. RT-PCR analysis was performed on myocardial samples (n=1 Healthy, n=5 IHD, n=5 DCM) to quantify the relative expression of **B**) cardiac stress markers (NPPA, NPPB) and **C**) alternative splicing isoform ratios for *TNNT2* (TnT4/TnT3) and *SCN5A* (isoform C/isoform A).

Recapitulating Pathological Splicing in vitro



A-**B**) UMAP projections of single cells isolated from (**A**) control and (**B**) fibrotic EHTs. Subclusters were identified as cardiomyocytes (red), cardiac progenitors (green), and fibroblasts (blue), with feature plots confirming canonical marker gene expression for each cell type. **C**) RT-PCR analysis of whole EHT lysates for cardiac stress markers (*NPPA*, *NPPB*) and alternative splicing isoform ratios for *TNNT2* and *SCN5A*. Data are means ± SD. *p<0.05.

Conclusions

- A novel 3D *in vitro* model of cardiac fibrosis was established using hiPSC-derived matrikines to replicate key features of the diseased heart microenvironment.
- The fibrotic microenvironment may directly trigger pathological splicing switches in cardiomyocytes, like the *TNNT2* and *SCN5A* isoform shift.

Resources

[1] Martino F., et al., Sci Transl Med, 14(672), 2022. PMID: 36417487

Graphical elements were created with BioRender.com

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