Engineering liver fibrosis in a three-dimensional, extracellular matrix-hydrogel disease model

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BACKGROUND

Modeling fibrosis requires replicating the mechano-chemical cues of the extracellular matrix (ECM), including binding sites, three-dimensionality, and biomechanics.

Aim: To engineer a liver fibrosis model based on native, decellularized ECM with spatial and compositional integrity, layered on a hydrogel of poly(ethylene glycol) diacrylate (PEGDA) that provides tunable mechanical properties. The model aims to support cell recolonization and facilitate the investigation of cell-matrix interactions, fibrogenic remodeling, and therapeutic screening in a physiologically relevant context.

Model design

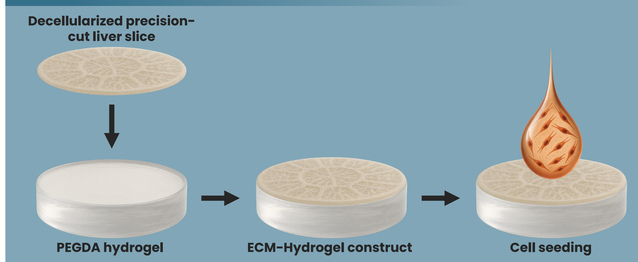

Fig. 1: Using a microtome, porcine liver tissue is precision-sliced and subsequently decellularized to isolate the ECM, retaining its architecture. The decellularized ECM slice is then mounted and fixed onto a hydrogel of PEGDA with mechanical properties tunable by the polymer concentration. The final construct is then seeded with hepatic stellate cells (HSCs).
Figure visualization was assisted by ChatGPT (OpenAI) based on textual descriptions.

Methods

ECM-hydrogel constructs were seeded with HSCs and cultured with a fibrotic cocktail (FC) containing profibrotic growth factors and cytokines. Following culture, samples were stained with phalloidin and DAPI, then imaged using confocal and multiphoton microscopy. Culture supernatants were analyzed using nordicPRO-C1™, nordicPRO-C3™, nordicPRO-C6™, nordicC3M™, and nordicC6M™ assays. Mechanical properties were measured using the Pavone nanoindentation system (Optics11 Life). Cytotoxicity was assessed using the Cytotoxicity Detection Kit^{PLUS} (Roche).

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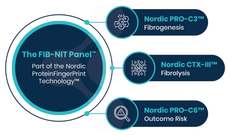

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Disclosures: FHS, AMG, MK, and DJL are employed at Nordic Bioscience. MK and DJL are shareholders.

Hepatic stellate cells colonize liver ECM

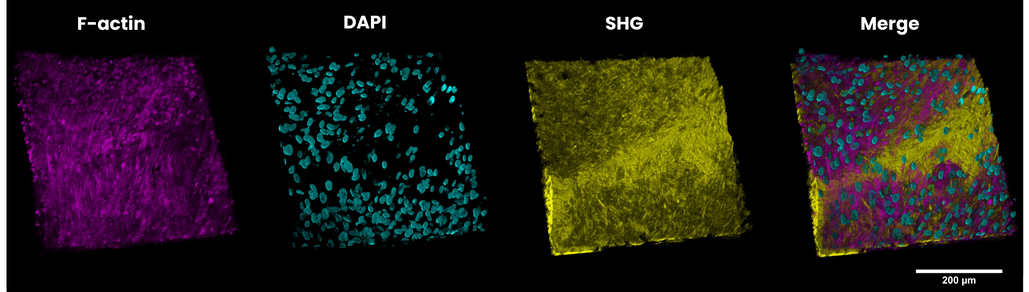

Fig. 2: Region of interest (ROI) showing HSC colonization of decellularized liver ECM.
Confocal microscopy visualizes F-actin stained with phalloidin-Alexa Fluor 594 and nuclei stained with DAPI. Multiphoton microscopy visualizes fibrillar collagens by second-harmonic generation (SHG). Images are presented as 3D projections of a z-stack with depth of 102.8 µm.

Model characterization

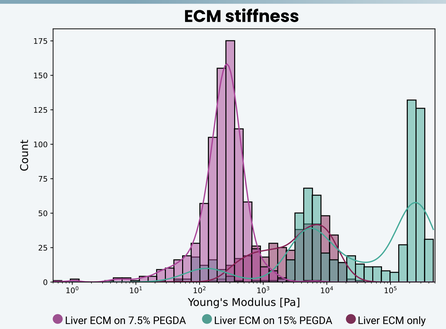

Fig. 3: Distribution of Young's Modulus measured in samples of liver ECM on 7.5% PEGDA, liver ECM on 15% PEGDA and liver ECM alone. Measurements were performed by Optics11 Life using their Pavone nanoindentation system.

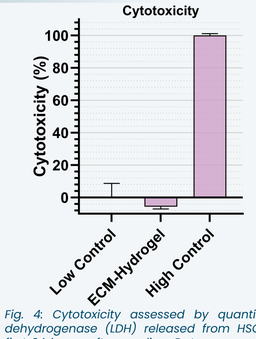

Fig. 4: Cytotoxicity assessed by quantifying lactate dehydrogenase (LDH) released from HSCs within the first 24 hours after seeding. Data were normalized to a low control (cells seeded on polystyrene) and a high control (cells lysed with Triton X-100).

Collagen formation

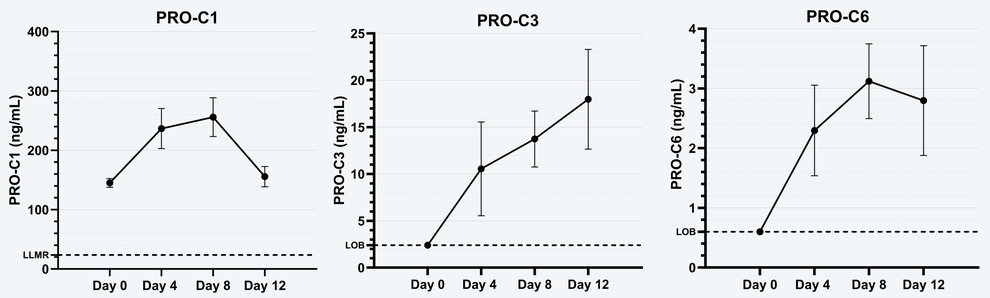

Fig. 5: Collagen type I formation was quantified using ELISA targeting PRO-C1.
LLMR: lower limit of measurement range.

Fig. 6: Collagen type III formation was quantified using ELISA targeting PRO-C3.
LOB: limit of blank.

Fig. 7: Collagen type VI formation was quantified using ELISA targeting PRO-C6.
LOB: limit of blank.

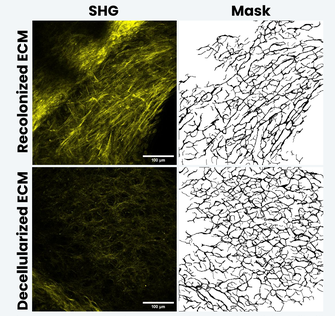

Fig. 8: Representative ROIs of SHG visualizing fibrillar collagens in recolonized ECM (top) and decellularized ECM (bottom). Masks of fibrillar collagens (right) were generated using the TWOMBLI plugin for ImageJ.

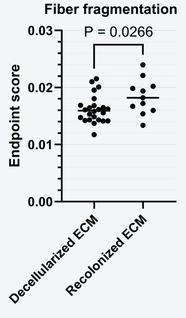

Fig. 9: Endpoints normalized to total fiber length per ROI using the masks of Fig. 8.

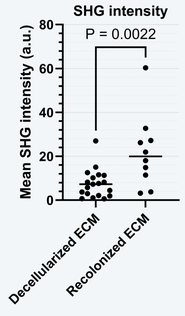

Fig. 10: Mean pixel intensity of SHG in ECM areas marked by the masks generated in Fig. 8.

Collagen degradation

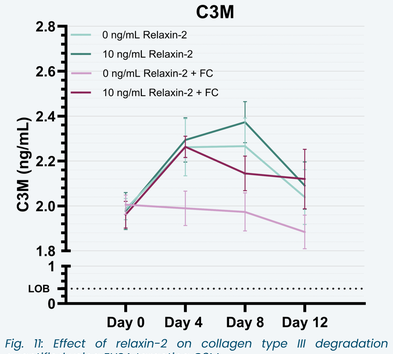

Fig. 11: Effect of relaxin-2 on collagen type III degradation quantified using ELISA targeting C3M.
FC: fibrotic cocktail. LOB: limit of blank.

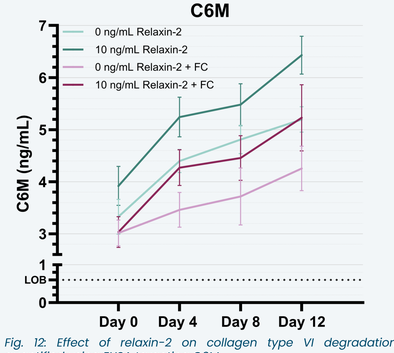

Fig. 12: Effect of relaxin-2 on collagen type VI degradation quantified using ELISA targeting C6M.
FC: fibrotic cocktail. LOB: limit of blank.

CONCLUSIONS

HSCs colonize porcine liver ECM and synthesize new collagen. Biochemical signaling and biomechanical properties drive ECM deposition, recapitulating key features of the fibrotic niche. Upon treatment with relaxin-2, HSCs display an ECM-degrading activity. The ECM-hydrogel constructs exhibit no cytotoxicity, and polymer concentration can be tuned to modulate the mechanical properties of the ECM. Due to its anatomical similarity to human tissue, porcine ECM offers a biologically relevant scaffold for *in vitro* fibrosis modeling without the ethical concerns associated with live animal use.