TissueSpec® Liver extracellular matrix cell culture substrate for 3D in-vitro models of NASH

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PHYSIOMIMETIC FIBROSIS MODEL

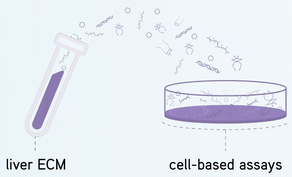
Problem: Current liver fibrosis models are not predictive

- Currently there are no drugs approved to treat non-alcoholic steatohepatitis (NASH).
- A major obstacle to develop effective treatments is the lack of predictive models for human steatohepatitis and liver fibrosis.
- Animal (e.g., rodent) models of NASH do not accurately recapitulate the natural history, histopathology, and metabolic profile of NASH.
- *In-vitro* NASH models have limited physiologic relevance and fail to provide the biochemical, structural, and mechanical environment in fibrotic human liver.

Solution: Physiomimetic approach to cell culture environments

- Xylyx Bio identifies and isolates the human disease environment, then develops and investigates **disease-specific ECM substrates** *in vitro* utilizing disease-relevant human cell types (e.g., hepatic stellate cells, hepatocytes).
- Cell phenotypes are compared against diseased human liver specimens with steatohepatitis/fibrosis prior to application in fibrotic liver disease models and anti-fibrotic drug testing

Xylyx Bio disease-specific ECM platform for drug development



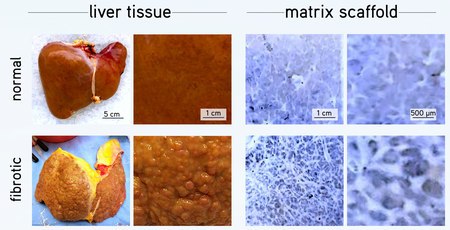
- Fibrosis-specific ECM substrates
- Applicable in 2D and 3D *in-vitro* models
- Xeno-free

- **Physiologically relevant substrates** contain the **suite of liver ECM proteins** & growth factors present in fibrotic human liver tissue.
- **Standardized experiments** are enabled by **consistent composition** across different lots, resulting in reproducible studies.
- **Accurate, predictive results** can be obtained due to **ideal *in-vitro* conditions** for maintaining cell phenotype.

XYLYX
the cell environment company

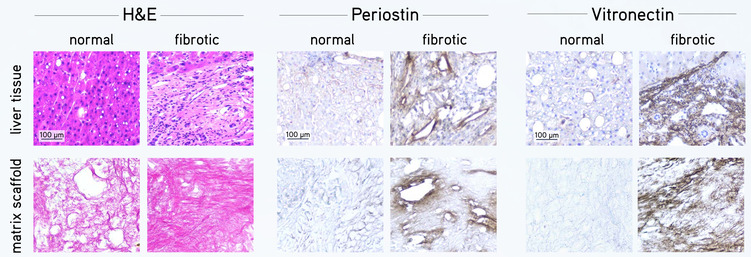
FIBROTIC LIVER ECM

ECM recapitulates normal or diseased human liver tissue *in vitro*



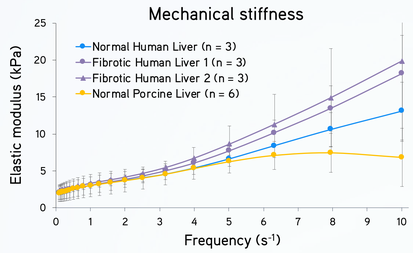
- **Methods:** Normal and fibrotic human livers were sectioned and washed to obtain **acellular human liver matrix**.

  H&E and immunostaining for fibrotic ECM proteins were performed on human liver tissues and matrix scaffolds to assess changes in fibrotic ECM structure/composition.



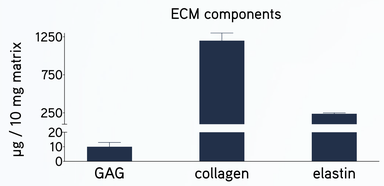
- **Results:** Acellular human liver matrix appeared translucent, with visible conduits throughout the matrix. H&E staining of fibrotic liver scaffolds showed presence of fibroconnective ECM, not observed in normal liver ECM. Immunohistochemical staining showed that fibrotic liver ECM scaffolds retain **increased fibrosis-associated ECM components periostin and vitronectin**.

Fibrotic liver ECM retains mechanics of fibrotic liver tissue



- **Methods:** Rheometric testing was conducted on hydrogels of normal and fibrotic human liver ECM.
- **Results:** Hydrogels of fibrotic liver ECM had higher elastic modulus than hydrogels of normal liver ECM, indicating that liver ECM hydrogels **mimic the mechanics of fibrotic human liver tissue in vitro**.

Liver ECM substrates have liver-specific biochemical composition

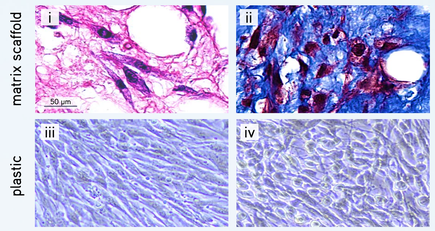


ECM component	Protein
collagens	type I, II, III, IV, V, VI
laminins	laminin γ1
glycoproteins	fibrillin 1, 2; mucin 5AC, 6
proteoglycans	heparan sulfate, hyaluronan
matrix-associated	albumin

- **Methods:** Biochemical assays were performed to quantify key ECM components collagens, elastin, and glycosaminoglycans (GAG) of swine liver ECM. Mass spectrophotometry (partial list) revealed detailed proteomic profile of liver ECM.
- **Results:** Normal liver ECM showed retention of ECM components in a **liver-specific ECM profile**, including 6 types of collagen, and multiple glycoproteins and proteoglycans known to regulate liver cell phenotype and function.

APPLICATIONS

Human hepatic stellate cells in human liver ECM scaffolds

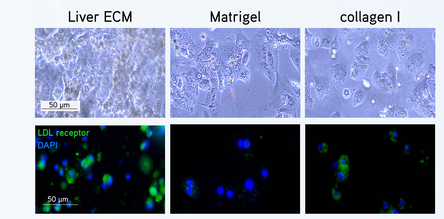


- **Methods:** Human hepatic stellate cells were cultured in human normal liver matrix scaffolds for 5 – 7 days, then fixed and stained with (i) H&E and (ii) trichrome.

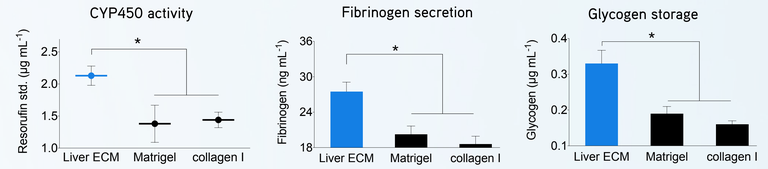
  Cell proliferation was quantified across 7 days by Alamar Blue assay. RNA was isolated after 7 days.
- **Results:** Hepatic stellate cells integrated into 3D human liver matrix scaffolds and showed fibroblastic phenotype (i,ii), compared to cells on collagen-coated plastic (iii) and plastic (iv).

  Hepatic stellate cells demonstrated: higher proliferation in human fibrotic liver scaffolds than normal liver scaffolds, activity **consistent with progressive hepatofibrotic disease**; and differential expression of fibrosis-related activation genes in liver scaffolds versus on plastic.

Human hepatocytes in swine liver ECM hydrogels



- **Methods:** Primary human hepatocytes or HepG2 cells were cultured for up to 7 days on normal swine liver ECM hydrogel, Matrigel, or collagen I hydrogel. Functional assessments were performed at day 5 using commercial assays.



- **Results:** Hepatocytes cultured on liver ECM hydrogels exhibit 3D structure formation and significantly higher hepatic functions: low-density lipoprotein uptake (green), cytochrome P450 (CYP1A2) activity, fibrinogen secretion, and glycogen storage, compared to Matrigel and collagen I gel. $*p < 0.05$. **Liver ECM hydrogel supports superior hepatocellular function**.
- **Conclusions:** Human liver ECM substrates comprise a highly physiologically relevant *in-vitro* NASH model that provides the biochemical and mechanical environment of diseased liver, and represents **a more predictive *in-vitro* model of human steatohepatitis and liver fibrosis**.

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We partner with leading pharma companies to further develop and integrate disease-specific ECM products into cell-based assays and established workflows to accelerate discovery and development. **For partnering opportunities, contact info@xylyxbio.com.**