Fondazione IRCCS Policlinico San Matteo

UNIVERSITÀ DI PAVIA

The Hepatic Matrisome: From Regenerative Hepatology to Drug Target and Biomarker Discovery

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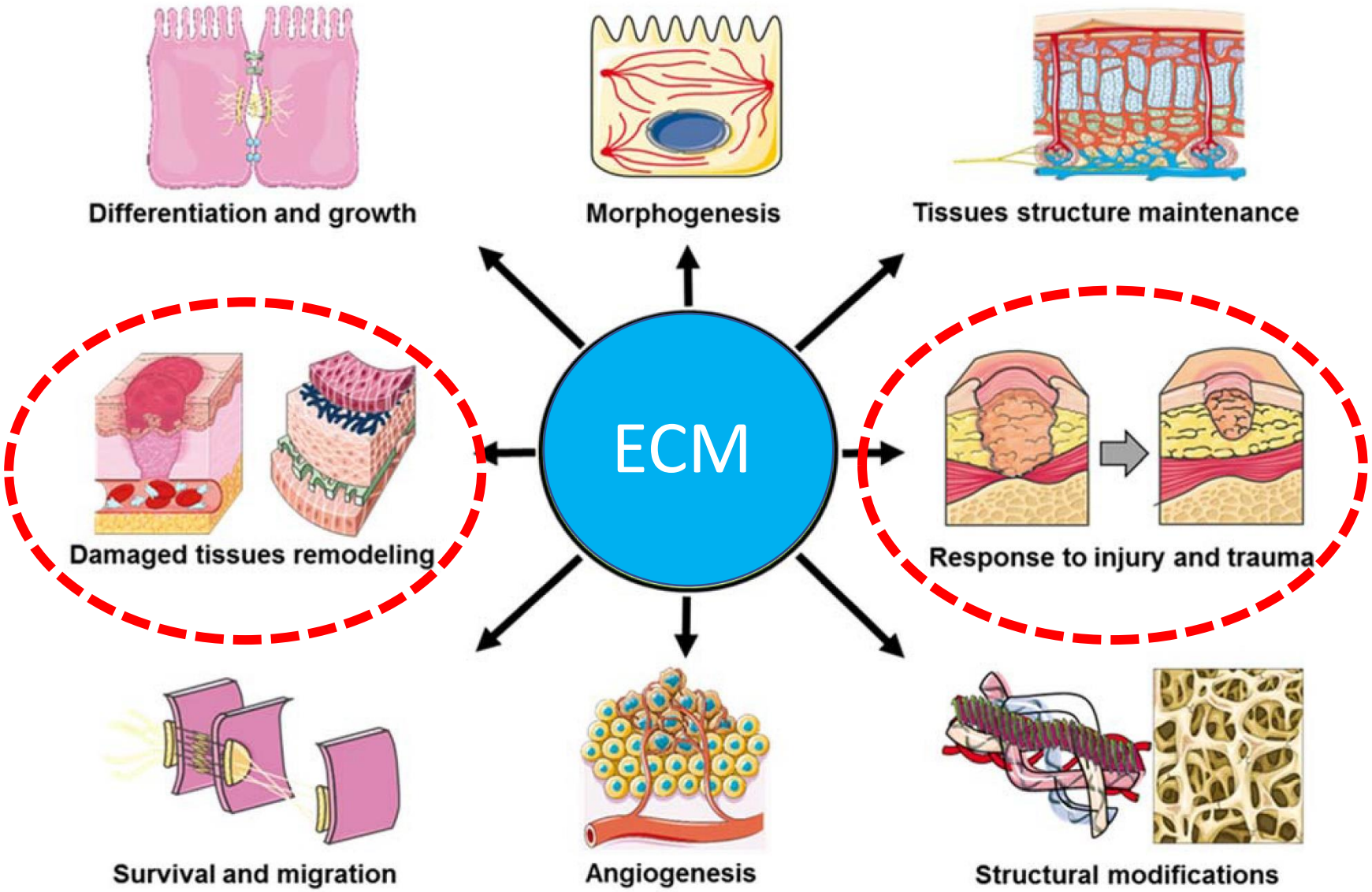
UCL

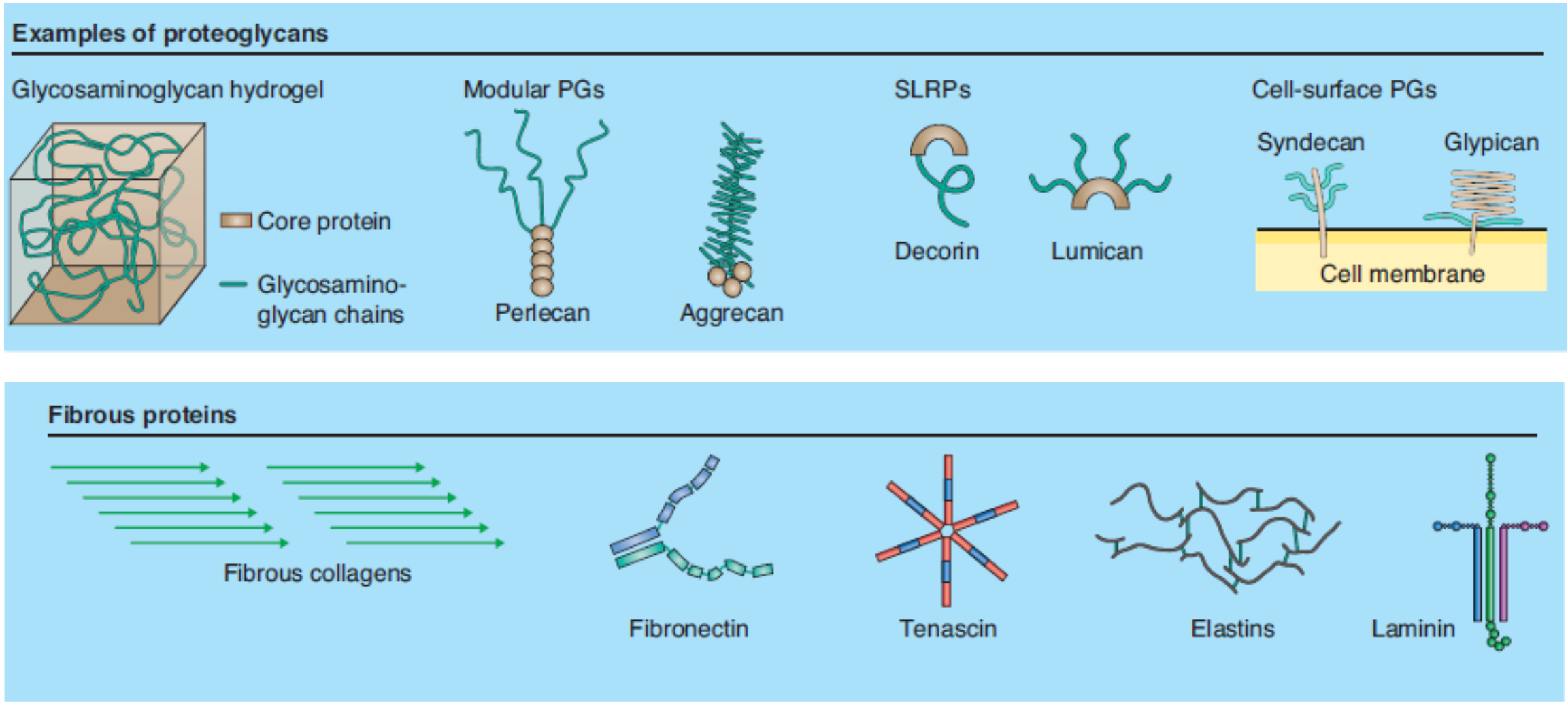
Royal Free London NHS
NHS Foundation Trust

NIHR | University College London Hospitals Biomedical Research Centre

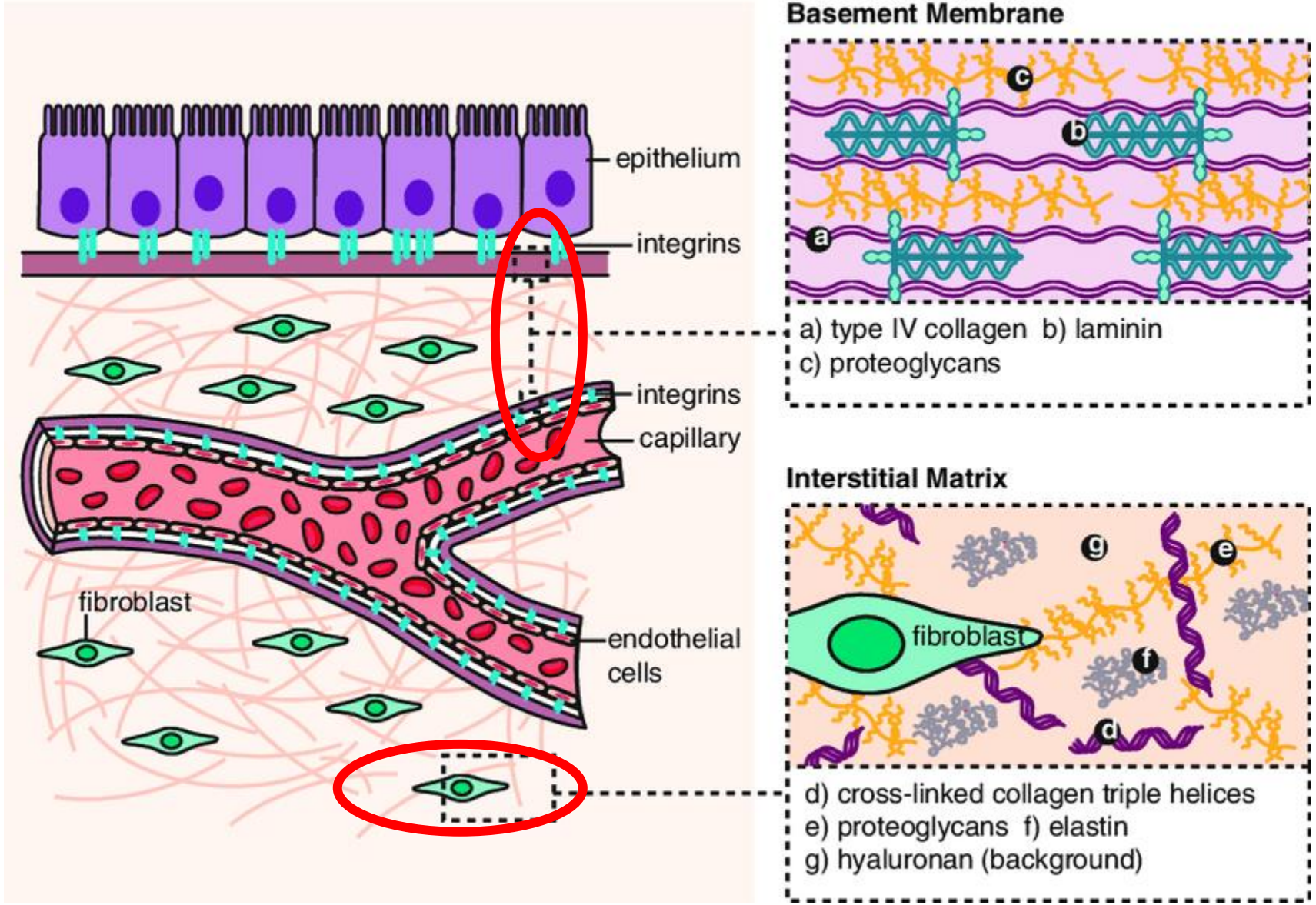
ECM Functions



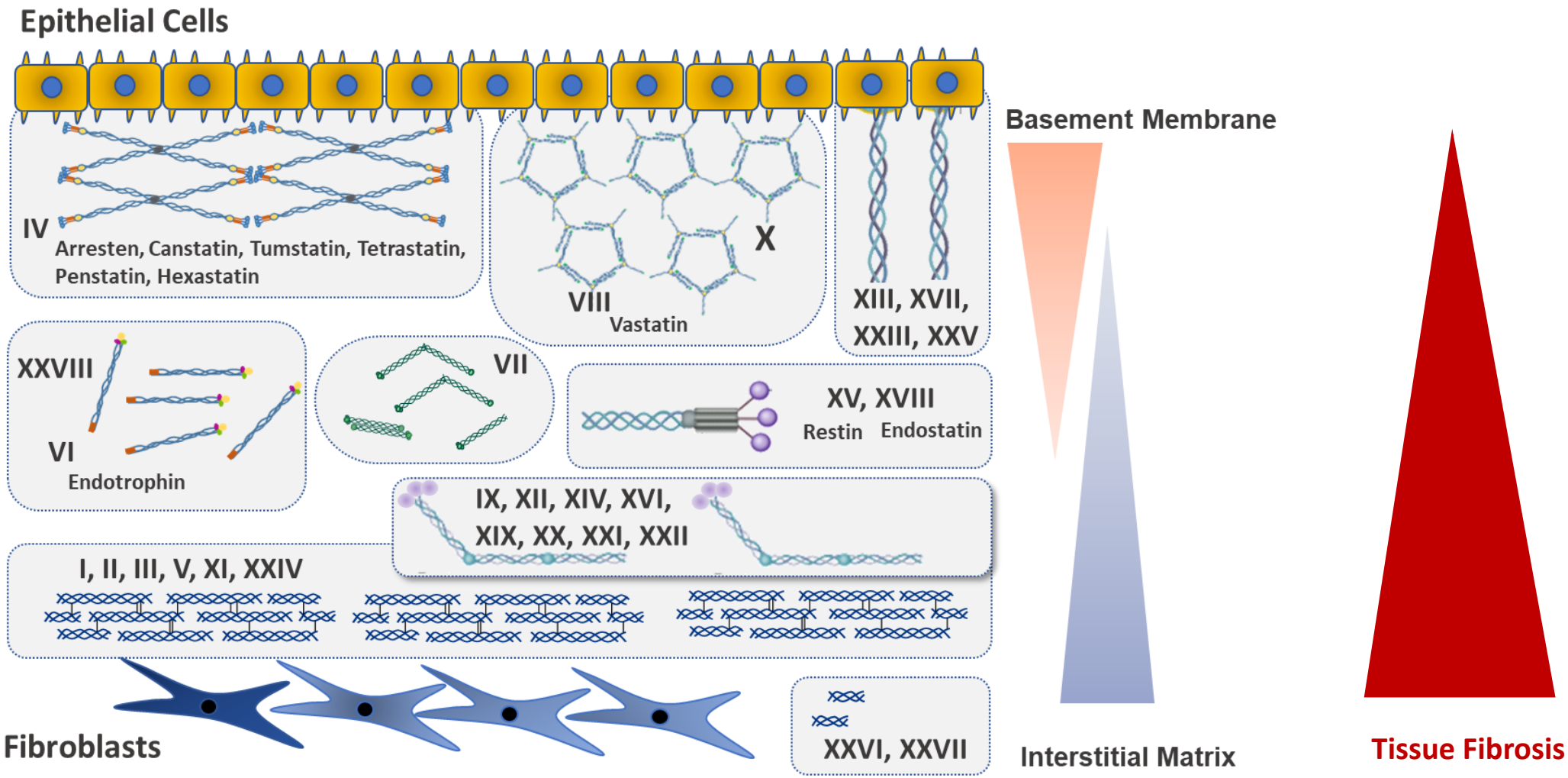
ECM Components



ECM: basement membrane and interstitial matrix

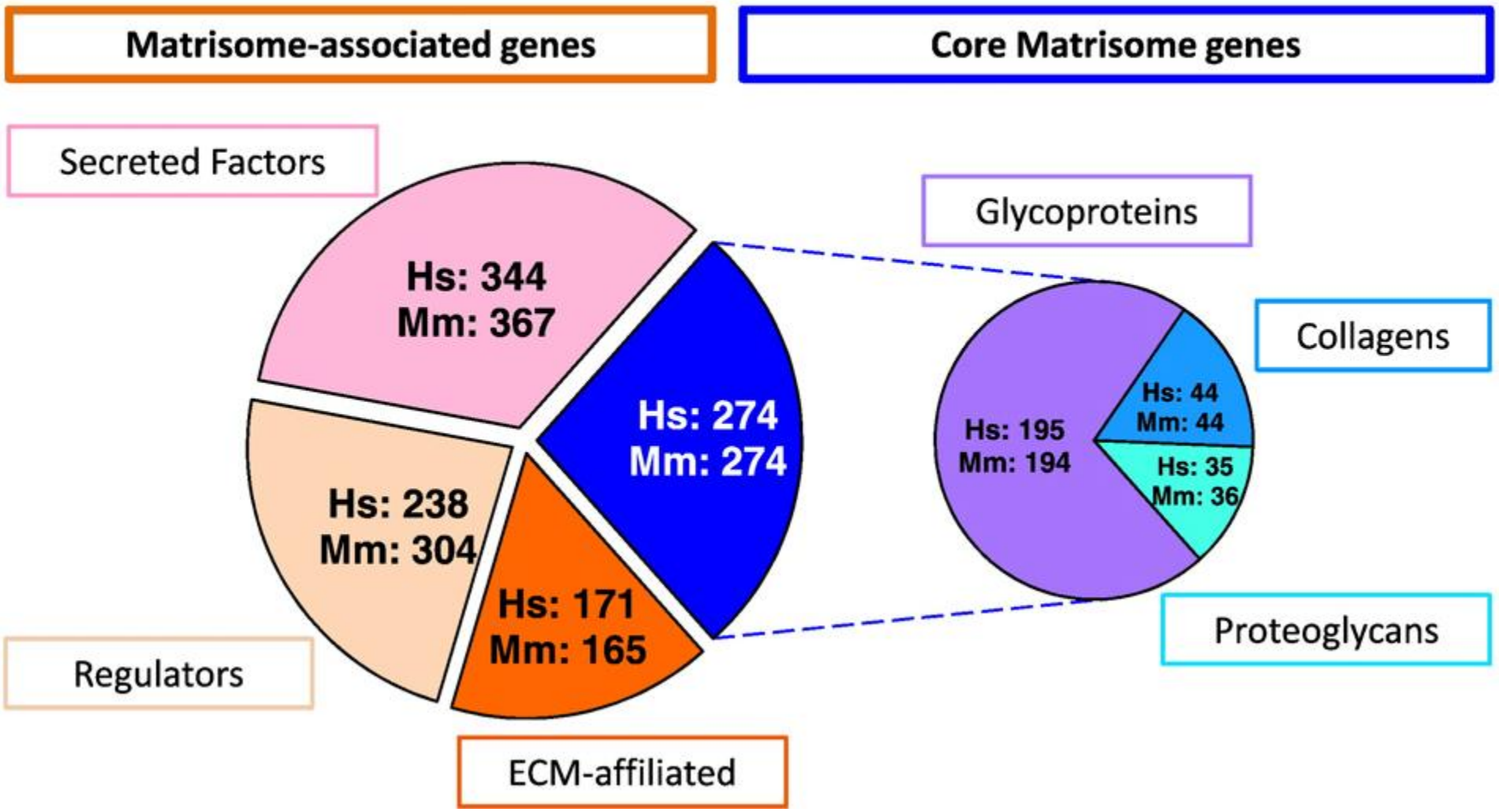


The 28 Collagens - Structure and Function



Karsdal et al., Hepatology, 2019

Matrisome


Matrisome-associated genes
Core Matrisome genes
Secreted Factors
Hs: 344
Mm: 367
Hs: 274
Mm: 274
Hs: 238
Mm: 304
Hs: 171
Mm: 165
Regulators
ECM-affiliated
Glycoproteins
Collagens
Proteoglycans
Hs: 195
Mm: 194
Hs: 44
Mm: 44
Hs: 35
Mm: 36

Matrisome

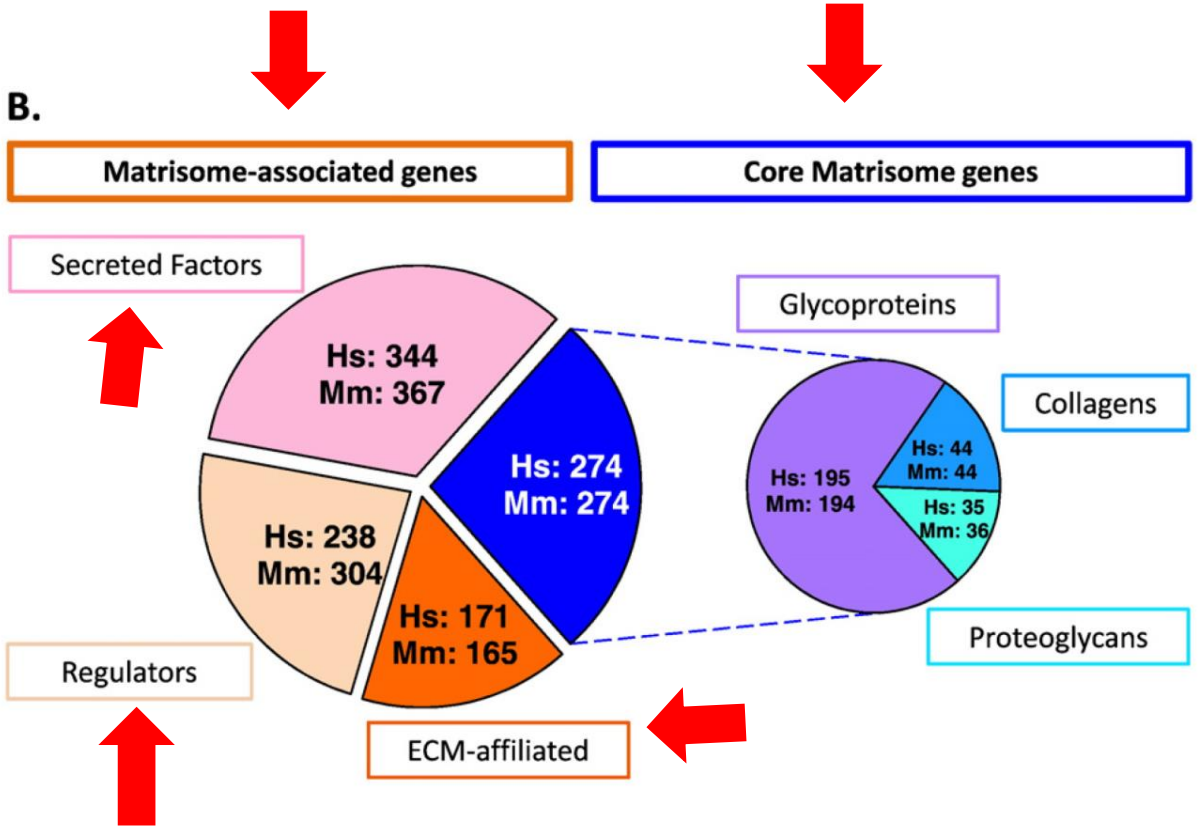
A.

1. Core Matrisome:
- ECM Glycoproteins: fibronectins, laminins, tenascins, thrombospondins, fibrillins, fibulins etc.
- Collagens (including transmembrane collagens)
- Proteoglycans

2. Matrisome-associated proteins

- ECM-affiliated proteins:
  - Proteins that may be considered as ECM proteins (e.g., mucins, C-type lectins, syndecans, glypicans)
  - Proteins viewed as secreted factors but which also associate with solid-phase complexes (e.g., semaphorins and their homologous receptors, plexins, collagen-related proteins and homologs)
  - Proteins that appear repeatedly in ECM-enriched preparations (e.g., annexins, galectins)
- ECM Regulators:
  - ECM-crosslinking (e.g.,lysyl oxidases, transglutaminases) and ECM-modifying enzymes (e.g.,sulfatases, extracellular kinases)
  - Proteases (e.g., MMPs, cathepsins…) and their inhibitors (e.g., TIMPs, cystatins)
- Secreted Factors (e.g., TGFβ, BMPs, Wnts, cytokines)

B.



Recent development of experimental techniques (e.g., tissue decellularization) have allowed the characterization of ECM composition by proteomic and other "omic" approaches to provide new insights into ECM biology

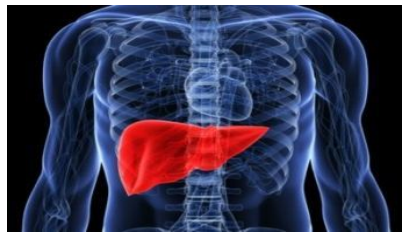
Development of Liver Scaffolds by Decellularization

Uygun et al. 2010
Whole Rat Liver

Yagi et al. 2013
Whole Pig liver

2010 — 2011 — 2013 — 2015 →

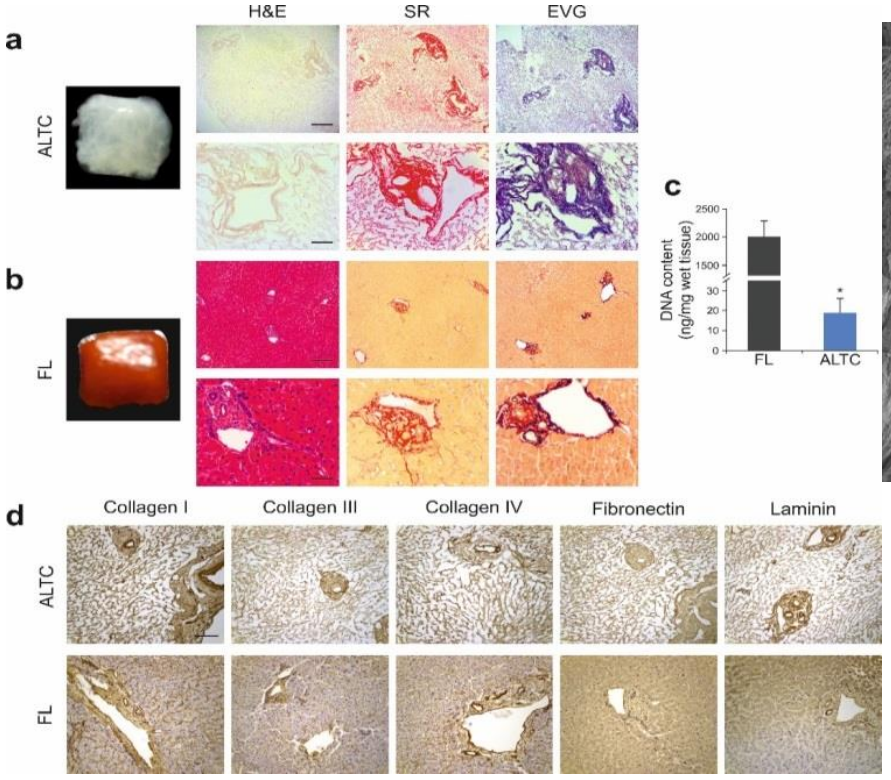
Baptista et al. 2011
Whole Ferret Liver

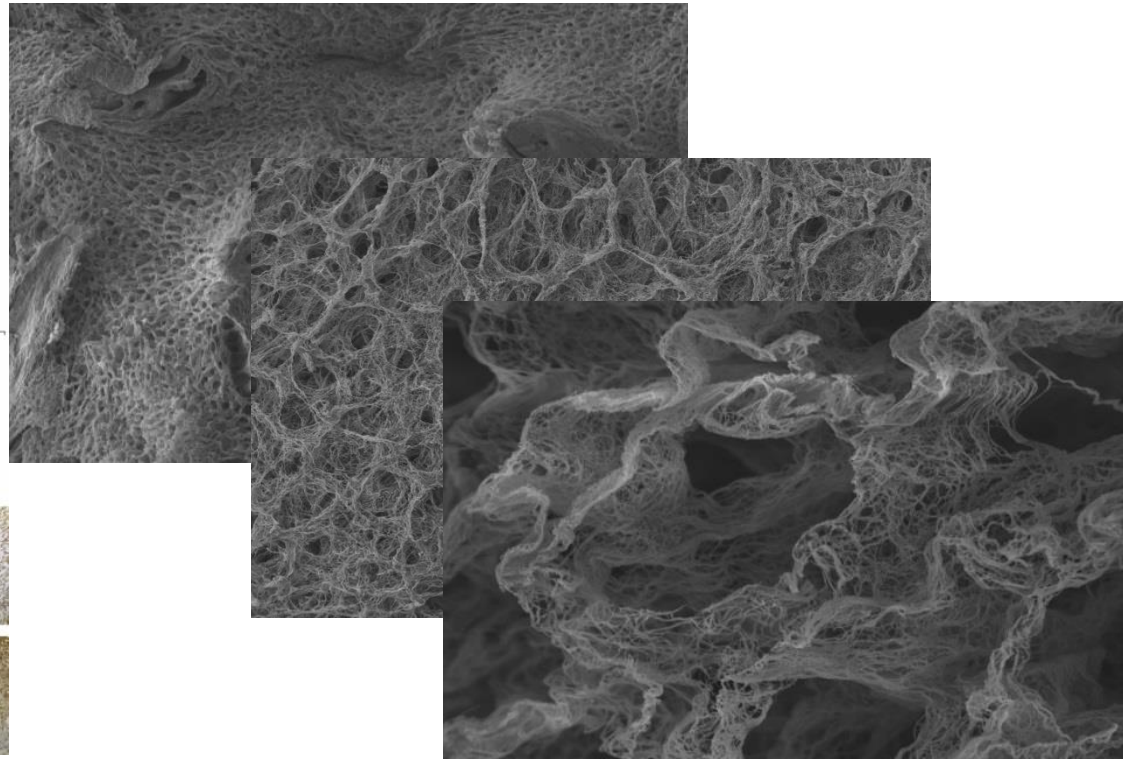


Mazza et al. 2015
Whole human Liver

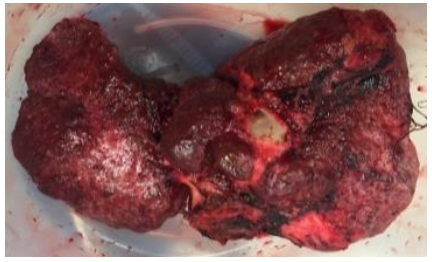
Lessons from Decellularized Human Liver

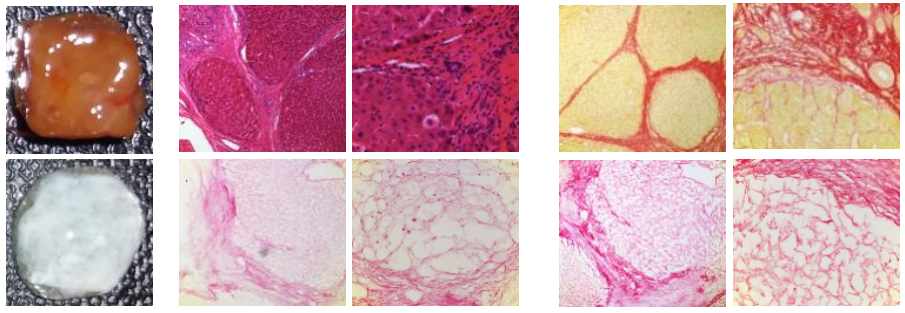
Donor healthy human liver

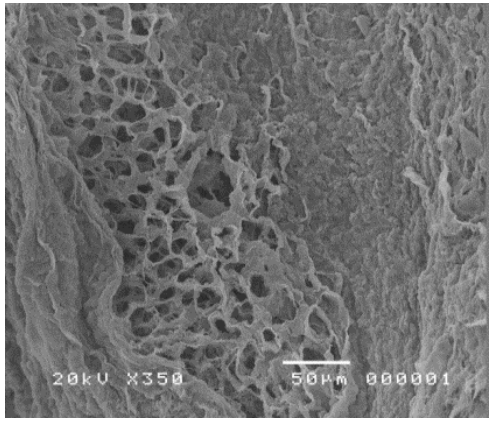


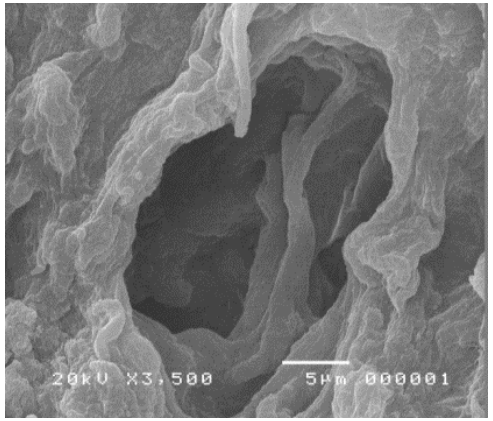


Explanted cirrhotic liver





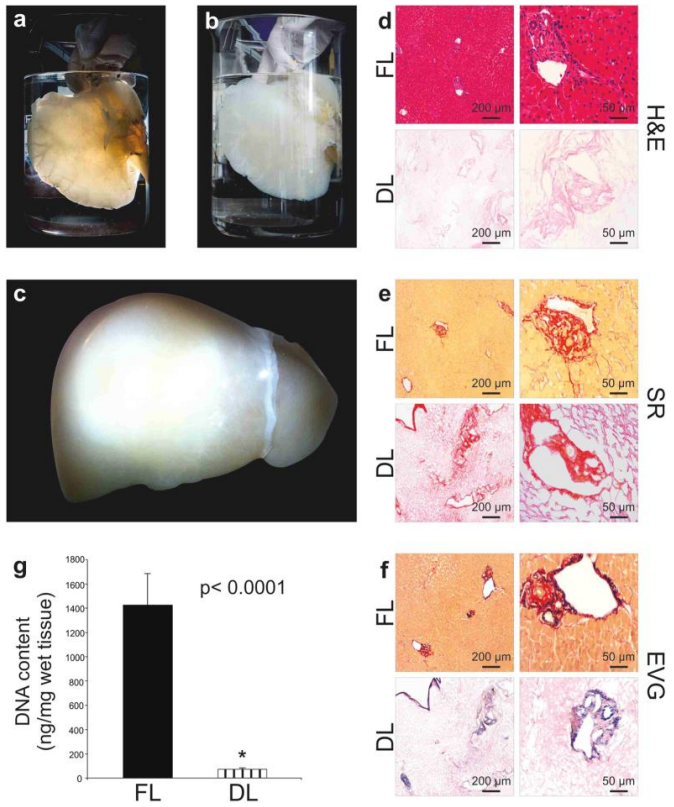




Mazza G et al, Scientific Report 2015; Mazza G et al, Scientific Report 2017

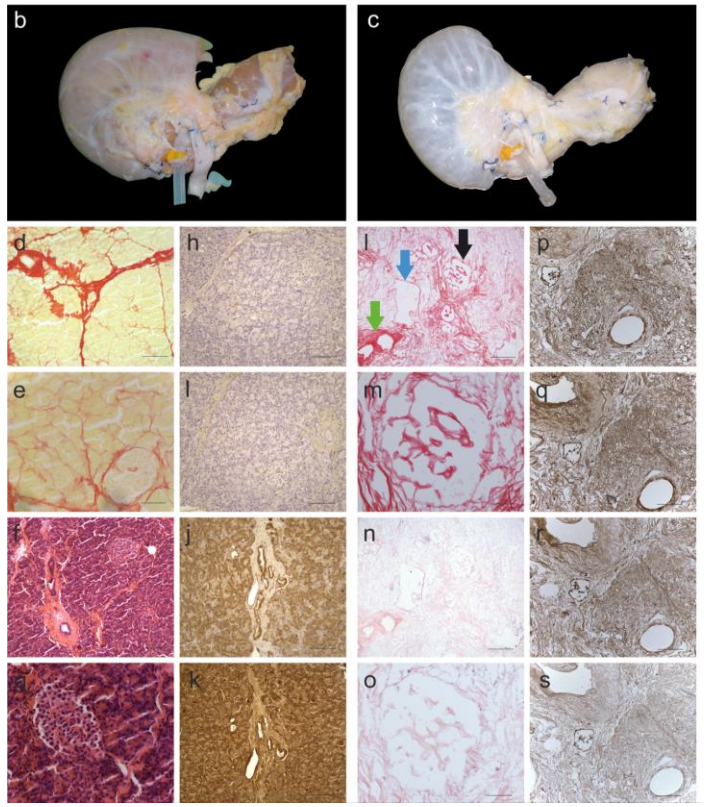
Human whole organ-decellularization

Healthy whole human liver



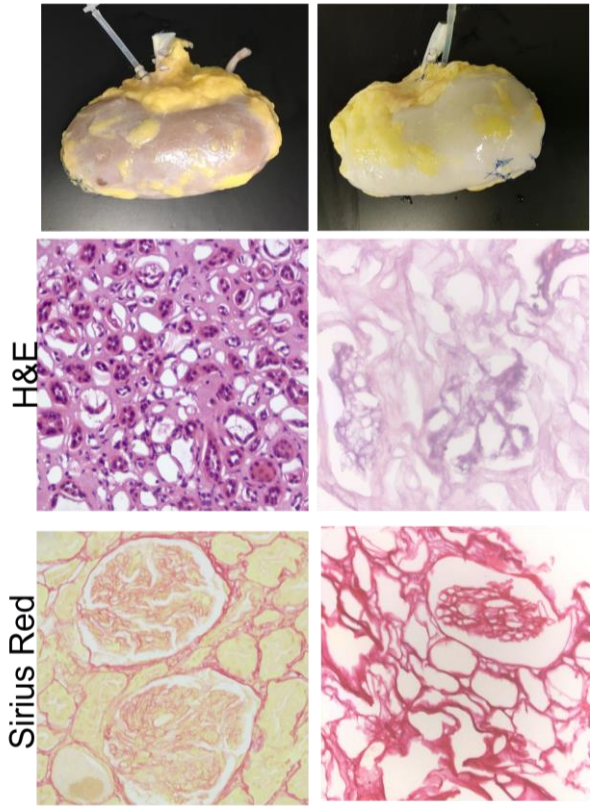
Mazza et al, Sci Rep (2015)*
* First ever human liver ECM publication

Healthy whole human pancreas



Al Akkad et al, Cells 2022

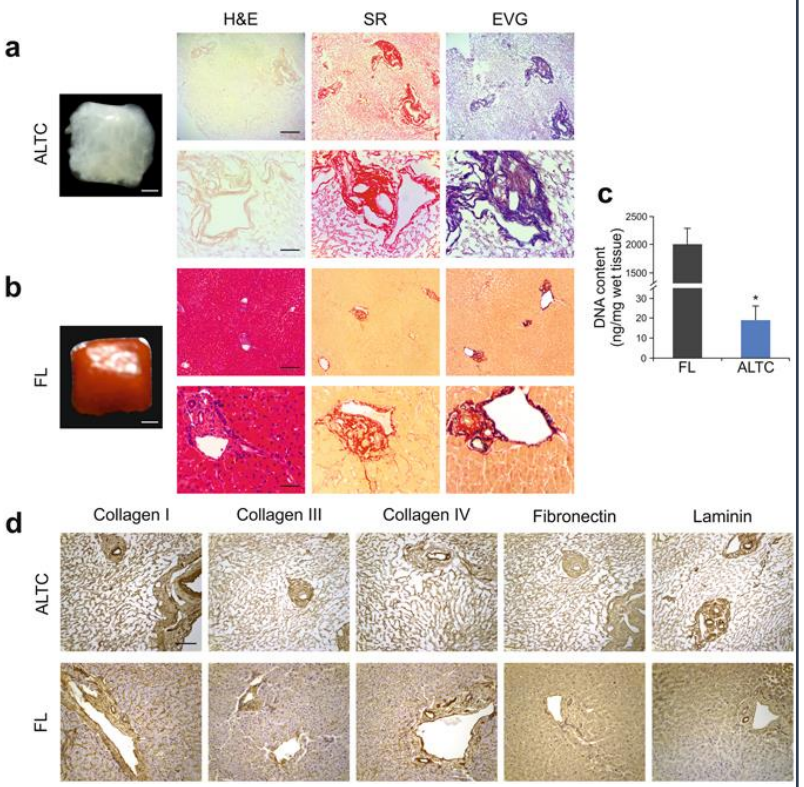
Healthy whole human kidney



Unpublished

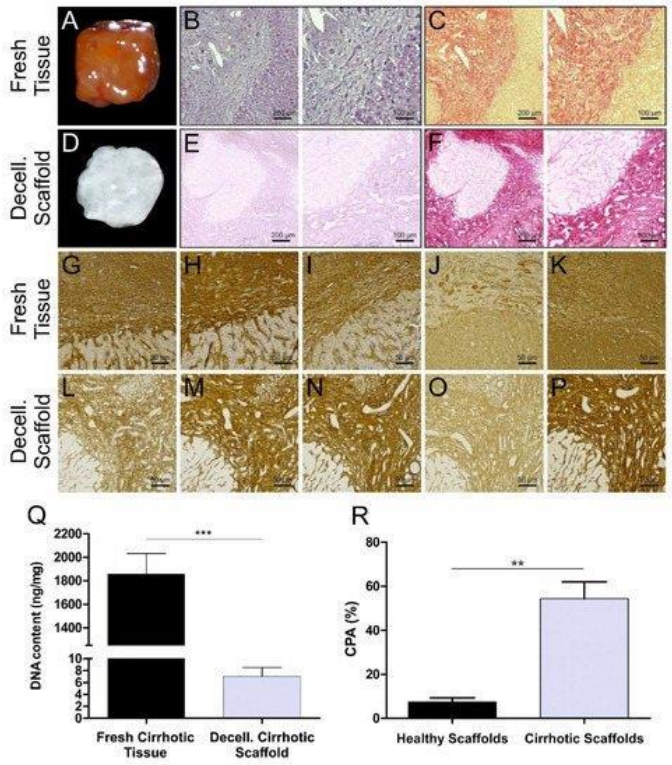
Human tissue agitation-decellularization

Healthy human liver



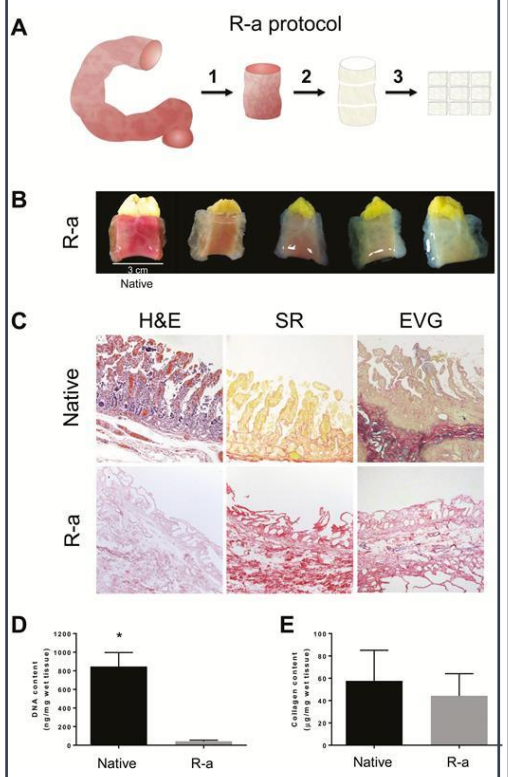
Mazza et al, Sci Rep (2017)

Cirrhotic human liver



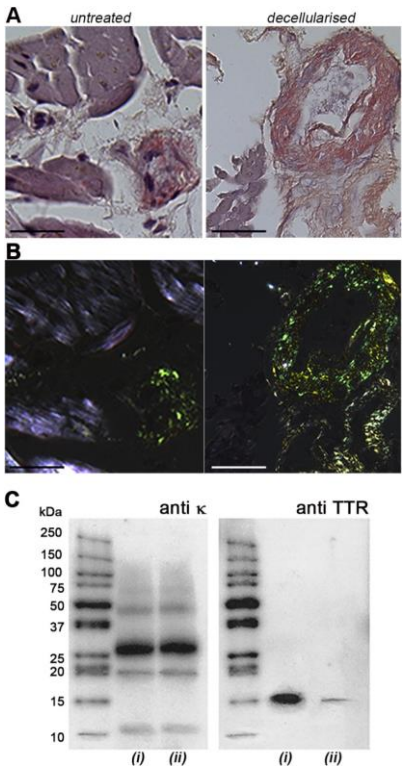
Mazza et al, Cells (2020)

Healthy human intestine



Giuffrida et al, Cells (2019)

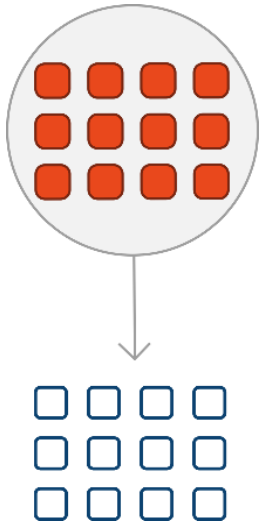
Amyloid human heart



Mangione, et al, J Proteomic (2017)

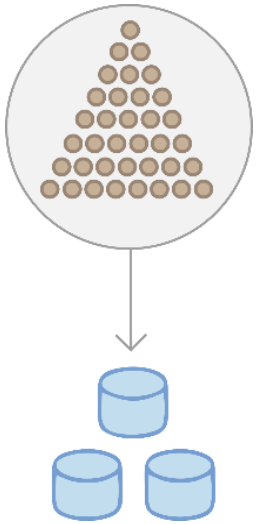
UCL Institute for Liver and Digestive Health

Our Platform Technology: Multiple Applications



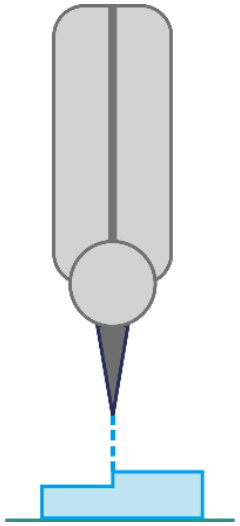
3D-DISEASE MODELLING

- ✓ Modelling of liver fibrosis, metastasis, liver and pancreatic cancer (incl. biomarkers)



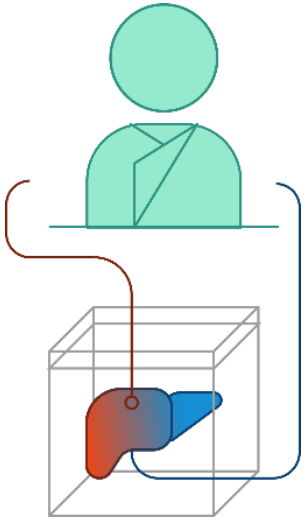
IMPLANTABLE LIVER TISSUE

- ✓ First-in-kind human ECM hydrogel
- ✓ Pre-clinical trials in rodents using iPSC or primary hepatocytes



BIO-INKS FOR 3D BIO-PRINTING

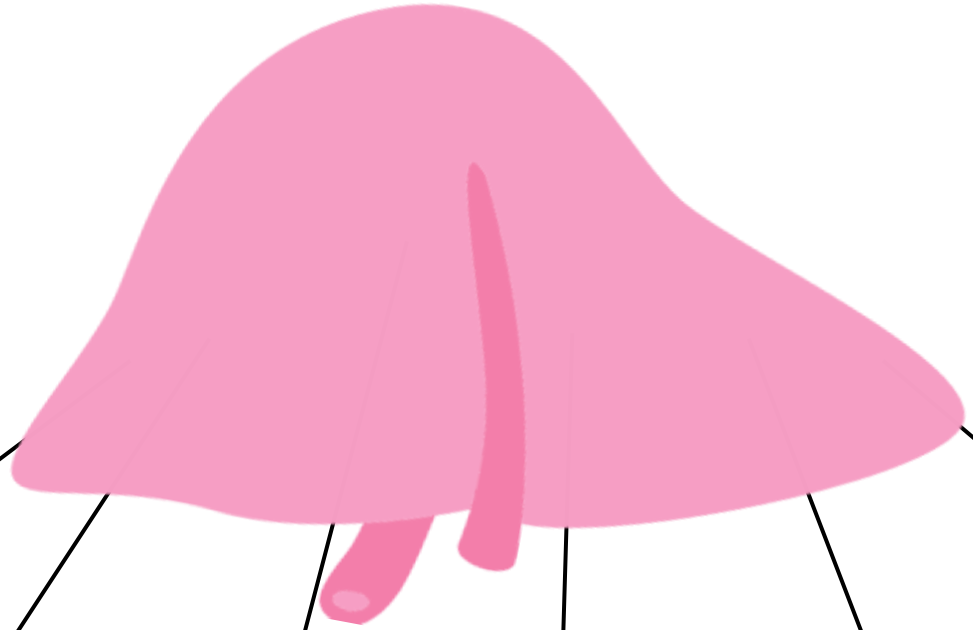
- ✓ First-in-kind human tissue specific



WHOLE ORGAN ENGINEERING

- ✓ First-in-kind human whole liver biological scaffolds
- ✓ R&D recellularization

Alternatives to Liver Transplantation (OLT)



"Split Liver"

Living Donor

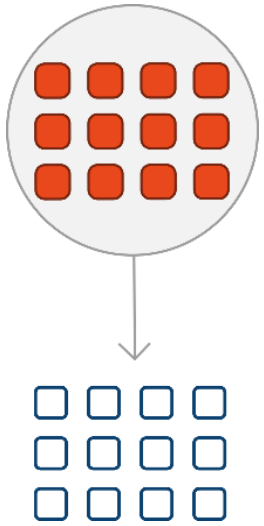
Partial OLT

Xenotransplantation

Hepatocyte transplantation

Bio-engineered Liver

Our Platform Technology: Multiple Applications



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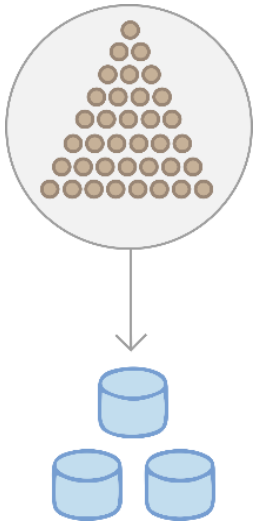

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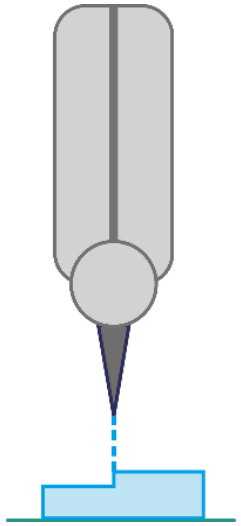

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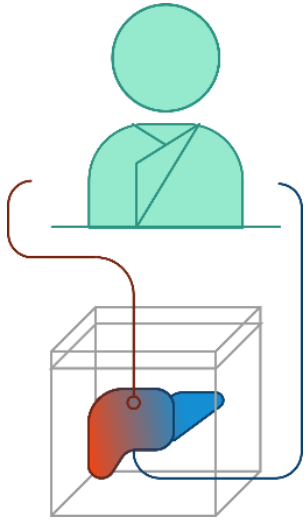

WHOLE ORGAN ENGINEERING

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- ✓ R&D recellularization

Clinical Use of Bio-engineered Hydrogels and Tissue Implants

Inborn Errors of Metabolism

Crigler-Najiar syndrome type 1
Familial hypercholesterolemia
Factor 7 deficiency
Glycogen storage disease type 1
Infantile Refsum disease
PFIC2
Urea cycle defects
- Ornithine transcarbamylase deficiency
- Arginosuccinate lyase deficiency
- Carbamoylphosphatase synthase type 1 deficit
- Citrullineamia

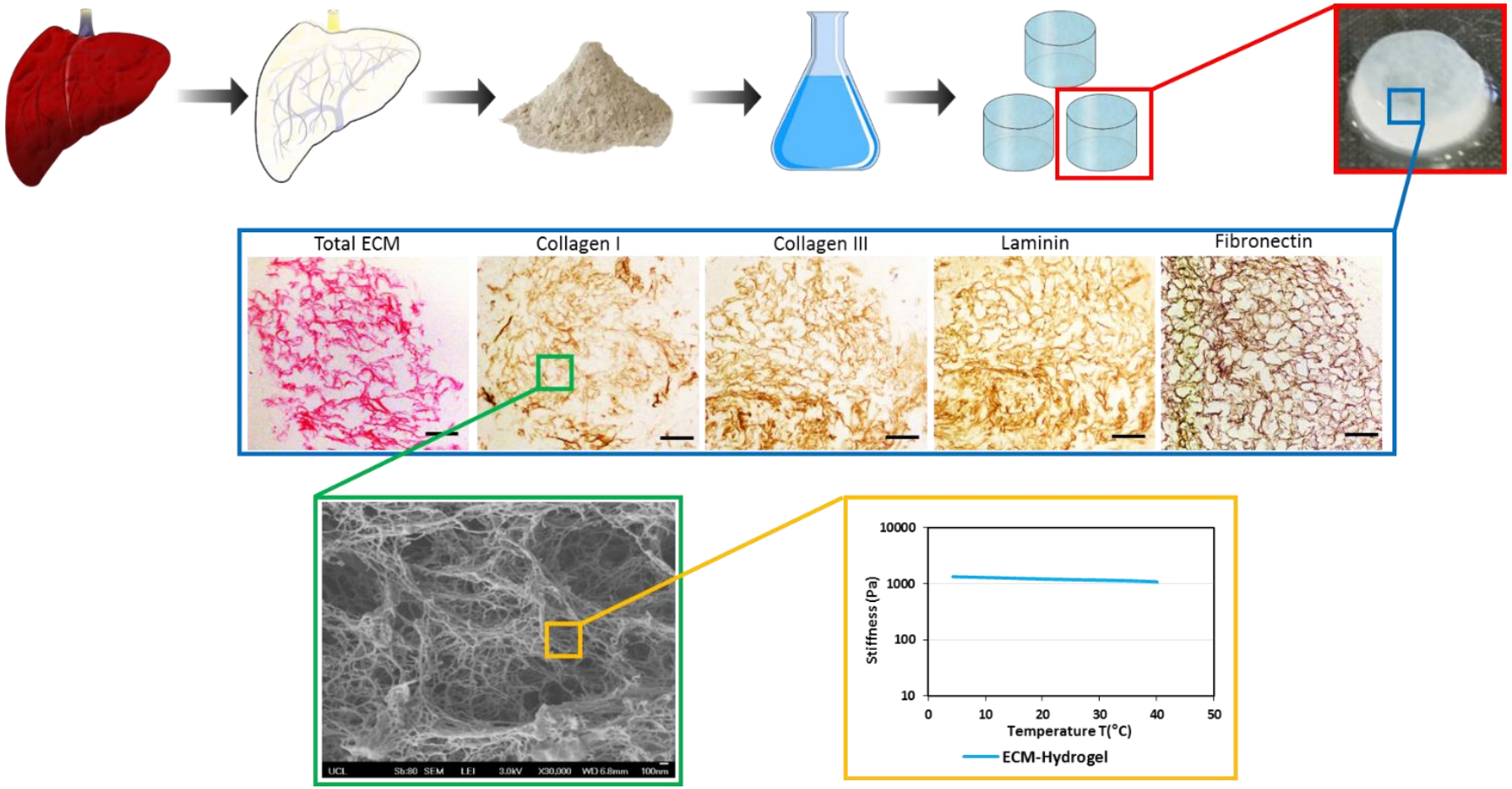
Acute Liver Failure

DILI
Viral
Mushroom poisoning
Post-surgical
Acute fatty liver of pregnancy

Acute on Chronic Liver Failure

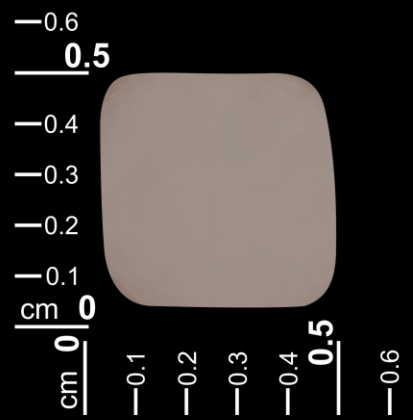
Alpha-1-antitrypsin deficiency
Alcoholic
Viral

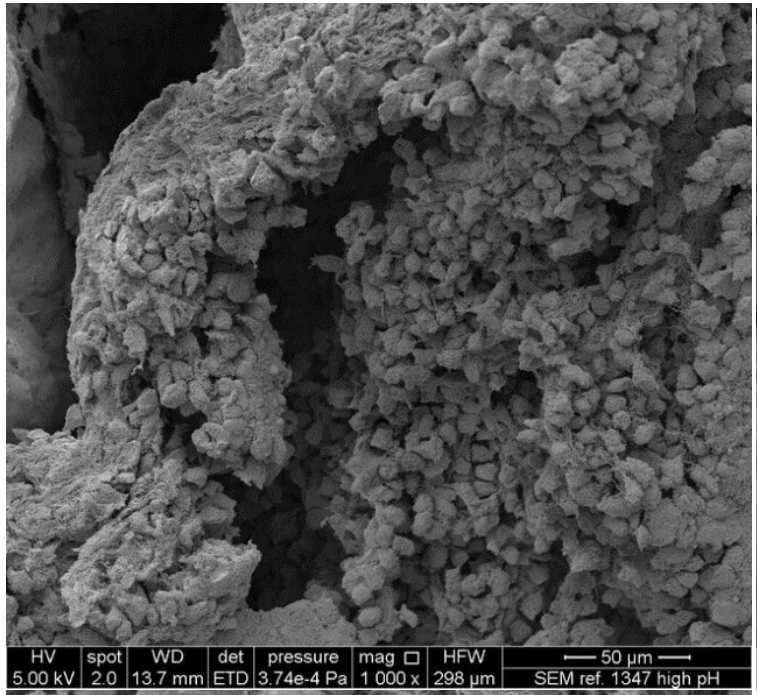
Human Liver ECM Hydrogel Characterization

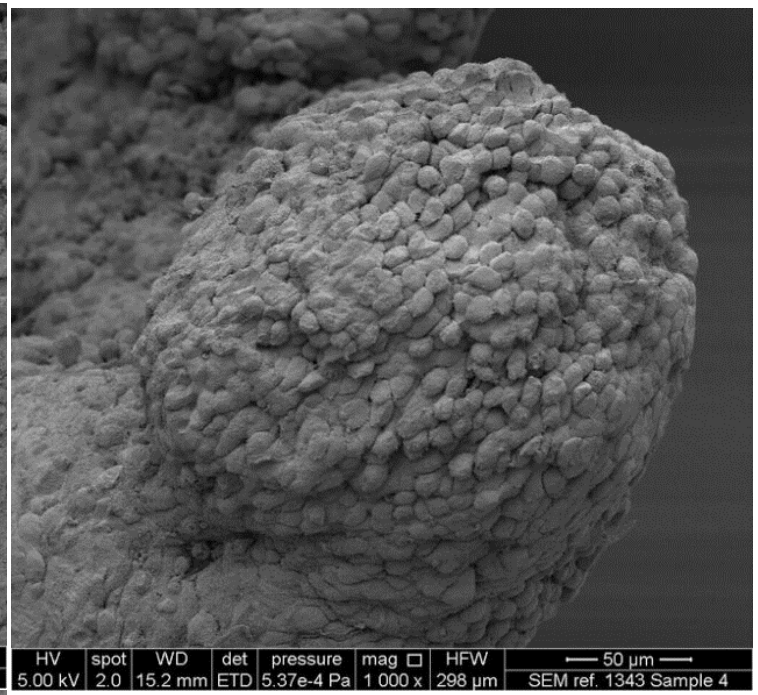


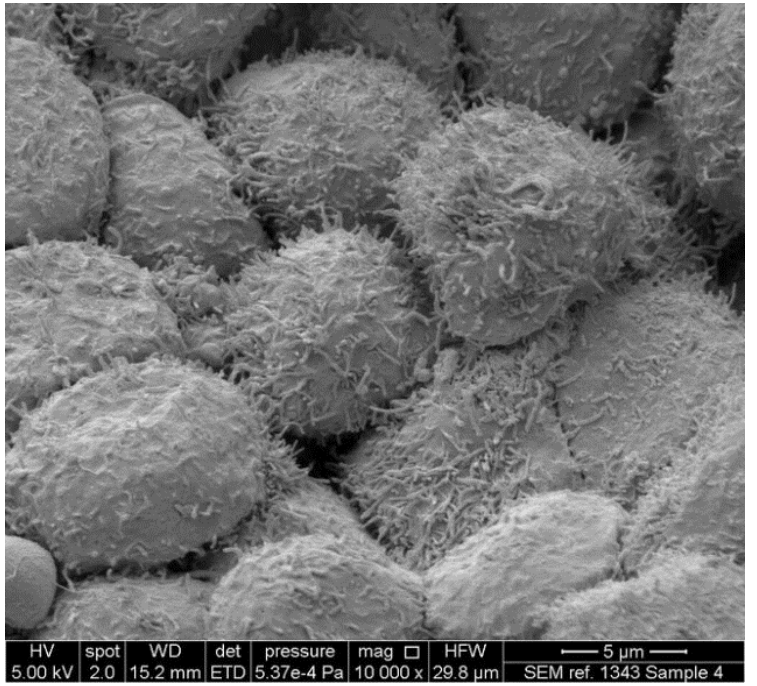
Human liver ECM hydrogels are derived from decellularized human liver using a protease-free step. Resultant hydrogels are characterized by preserved ECM proteins compositions, porous 3D architecture, tissue stiffness of 1kPa with tissue stiffness stability from 4^{o}C to 40^{o}C

From 3D ECM Scaffolds to Liver Bio-Ink

Decellularised Liver Scaffold	Liver ECM Hydrogel	Liver ECM Bioink
		
Implantable only	Injectable and implantable	Implantable only
Possess exact tissue-specific ECM composition and micro-architecture	Possess tissue-specific ECM composition	Possess tissue-specific ECM composition
Cells need time to migrate and accommodate	Cells need time to migrate and accommodate	Cells can be mixed homogeneously within the bioink
Low scalability	High scalability	High scalability
Mainly used for in vitro 3D models Fixed size of 5mmx5mmx5mm	>90% of ECM composition Challenges in mixing directly cells into the ECM	Tissue bioprinting: high control of spatial deposition ½ of ECM content vs liver ECM hydrogel


HV spot WD det pressure mag HFW
5.00 kV 2.0 13.7 mm ETD 3.74e-4 Pa 1 000 x 298 µm
50 µm
SEM ref. 1347 high pH


HV spot WD det pressure mag HFW
5.00 kV 2.0 15.2 mm ETD 5.37e-4 Pa 1 000 x 298 µm
50 µm
SEM ref. 1343 Sample 4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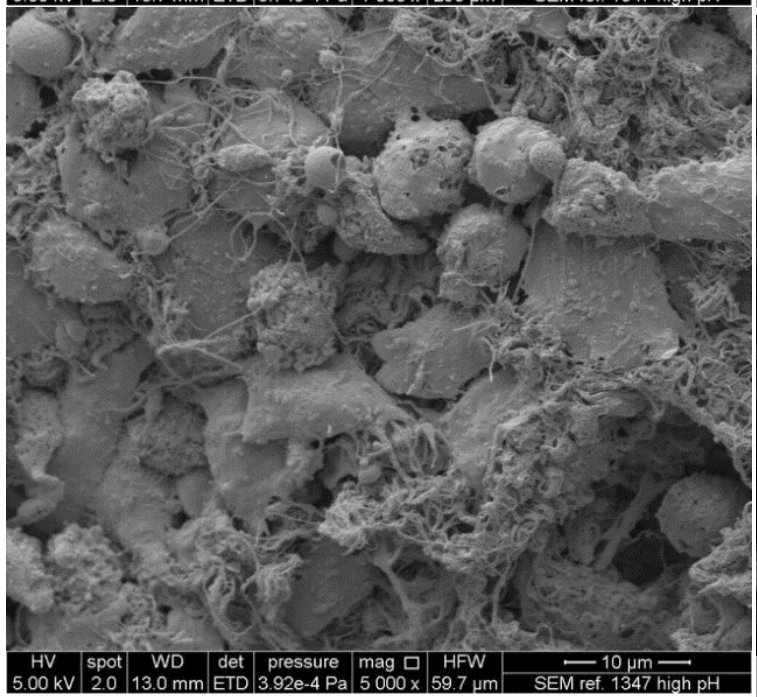
5 µm
SEM ref. 1343 Sample 4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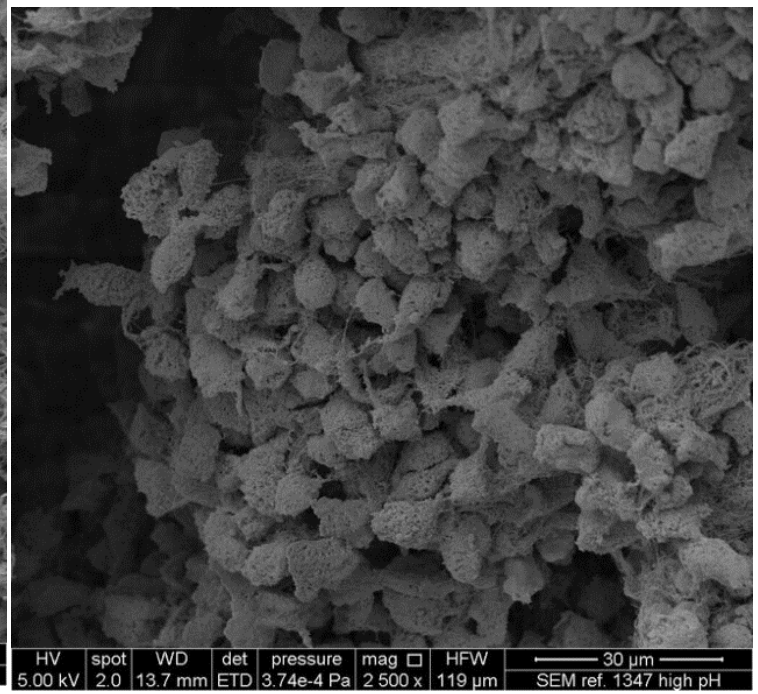
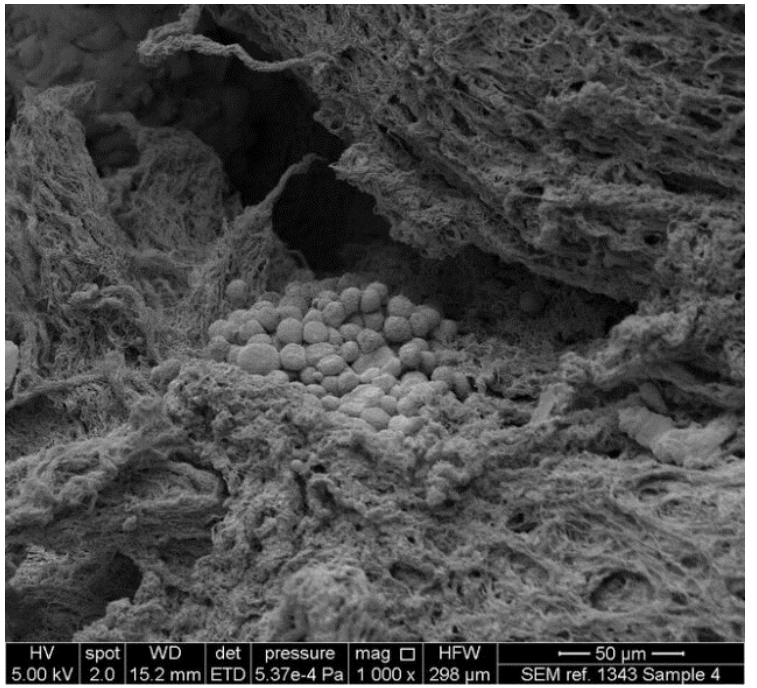
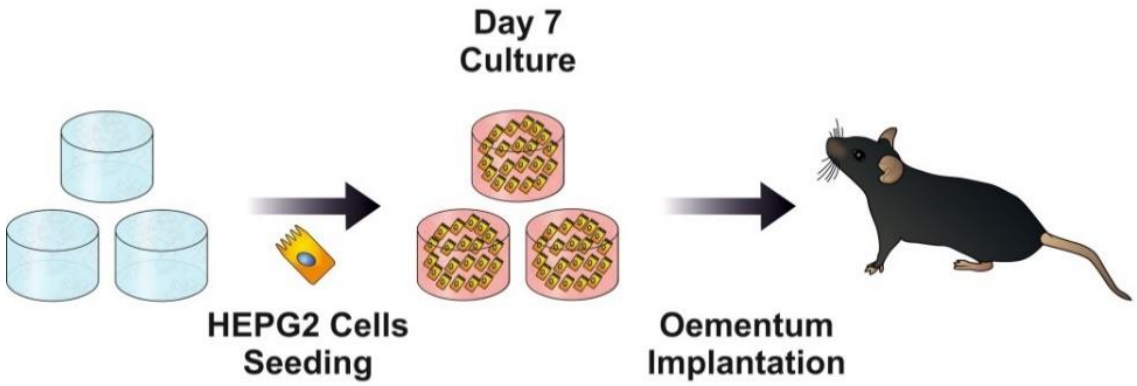
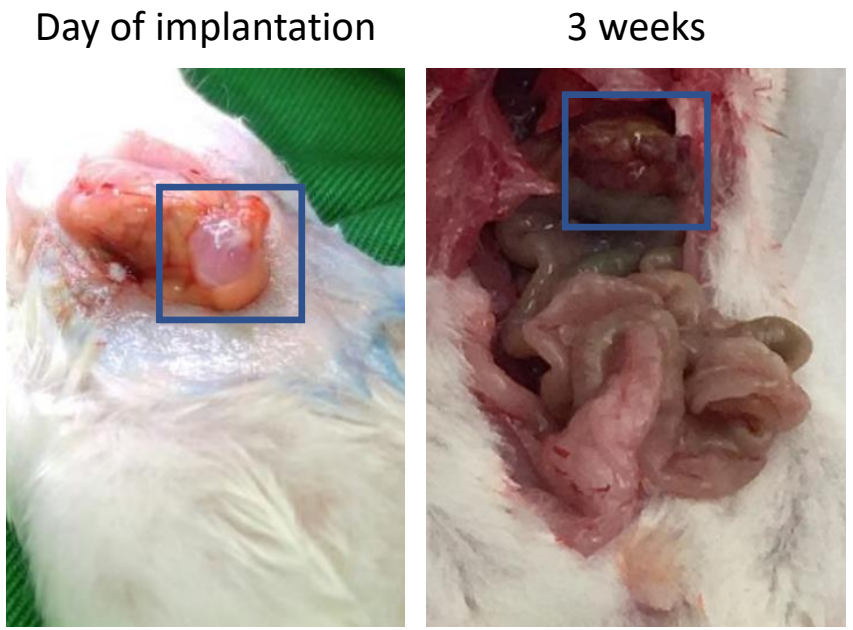
10 µm
SEM ref. 1347 high pH


HV spot WD det pressure mag HFW
5.00 kV 2.0 13.7 mm ETD 3.74e-4 Pa 2 500 x 119 µm
30 µm
SEM ref. 1347 high pH


HV spot WD det pressure mag HFW
5.00 kV 2.0 15.2 mm ETD 5.37e-4 Pa 1 000 x 298 µm
50 µm
SEM ref. 1343 Sample 4

Bio-engineered Human Liver ECM Hydrogel: Biocompatibility

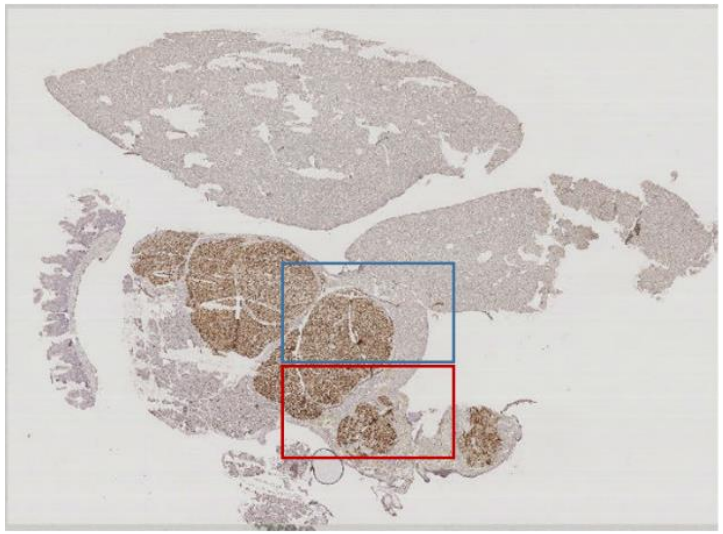


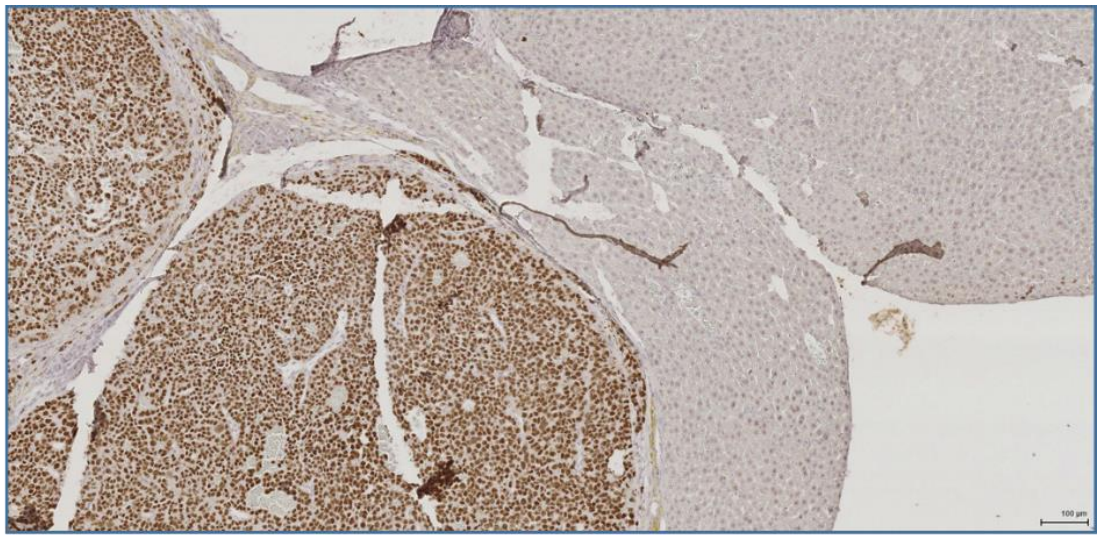


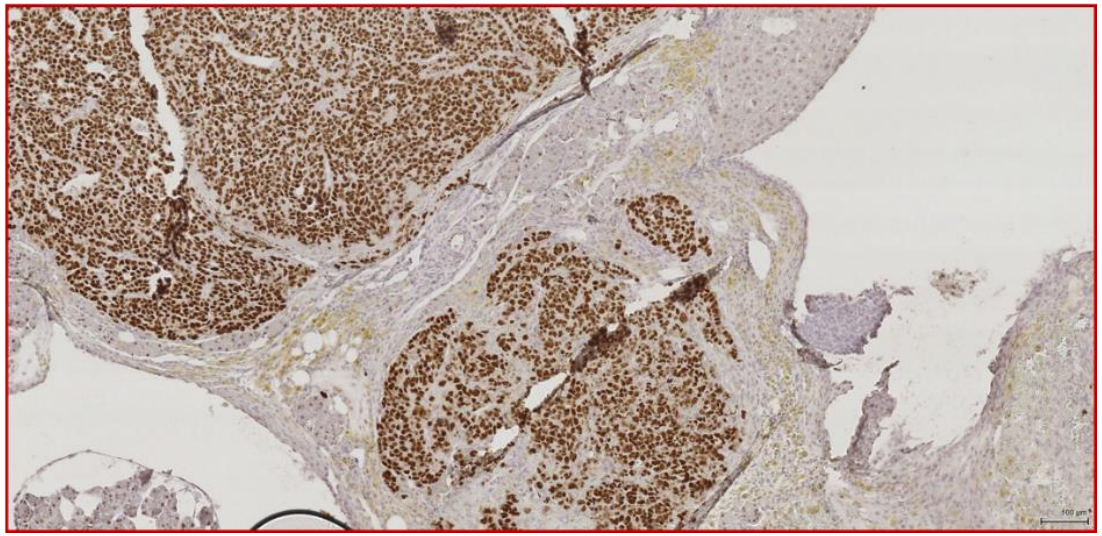
Human liver ECM hydrogels were reseeded with HepG2 for 7 days in vitro and ectopically implanted intra-omentum in immunodeficient mice for 3 weeks.

Bio-engineered Human Liver ECM Hydrogel: Biocompatibility

3 weeks: Peritoneum implantation site

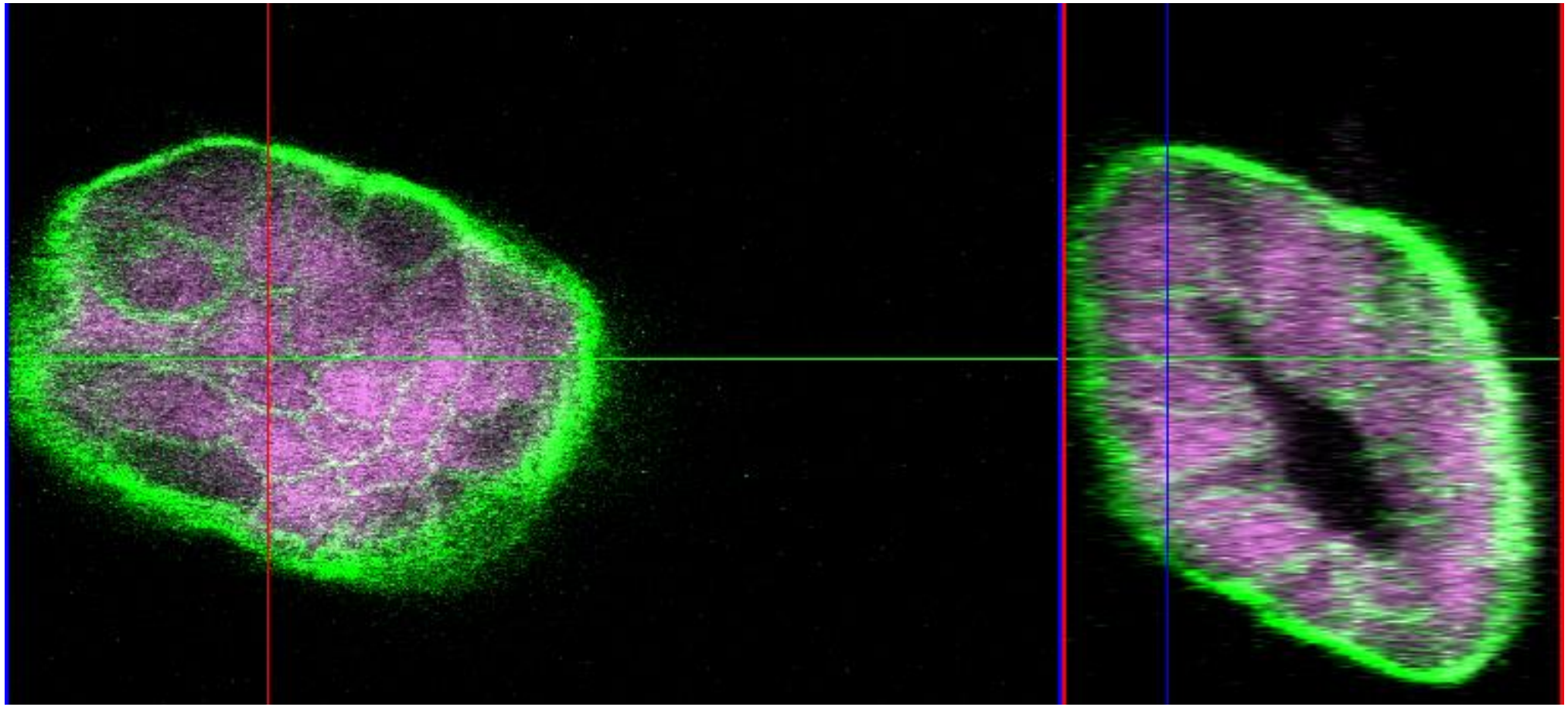




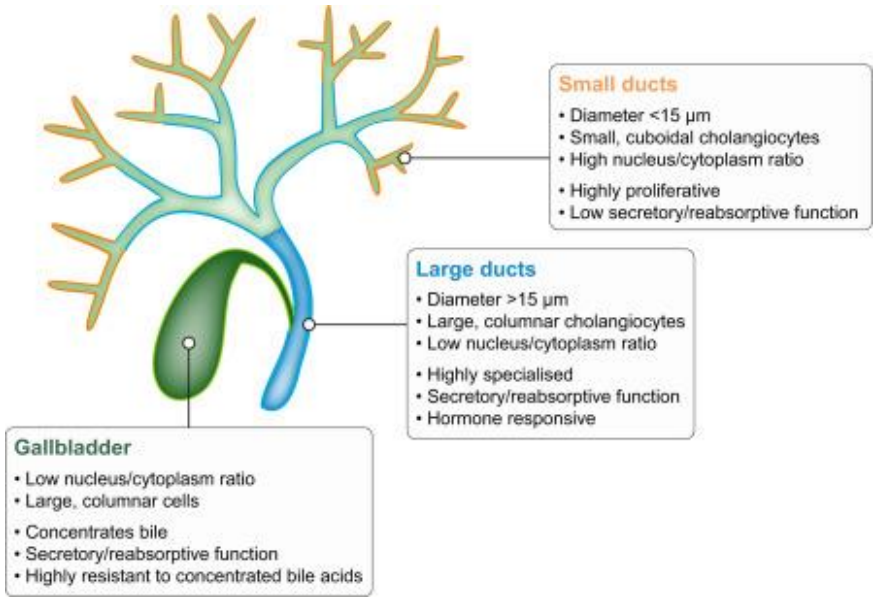


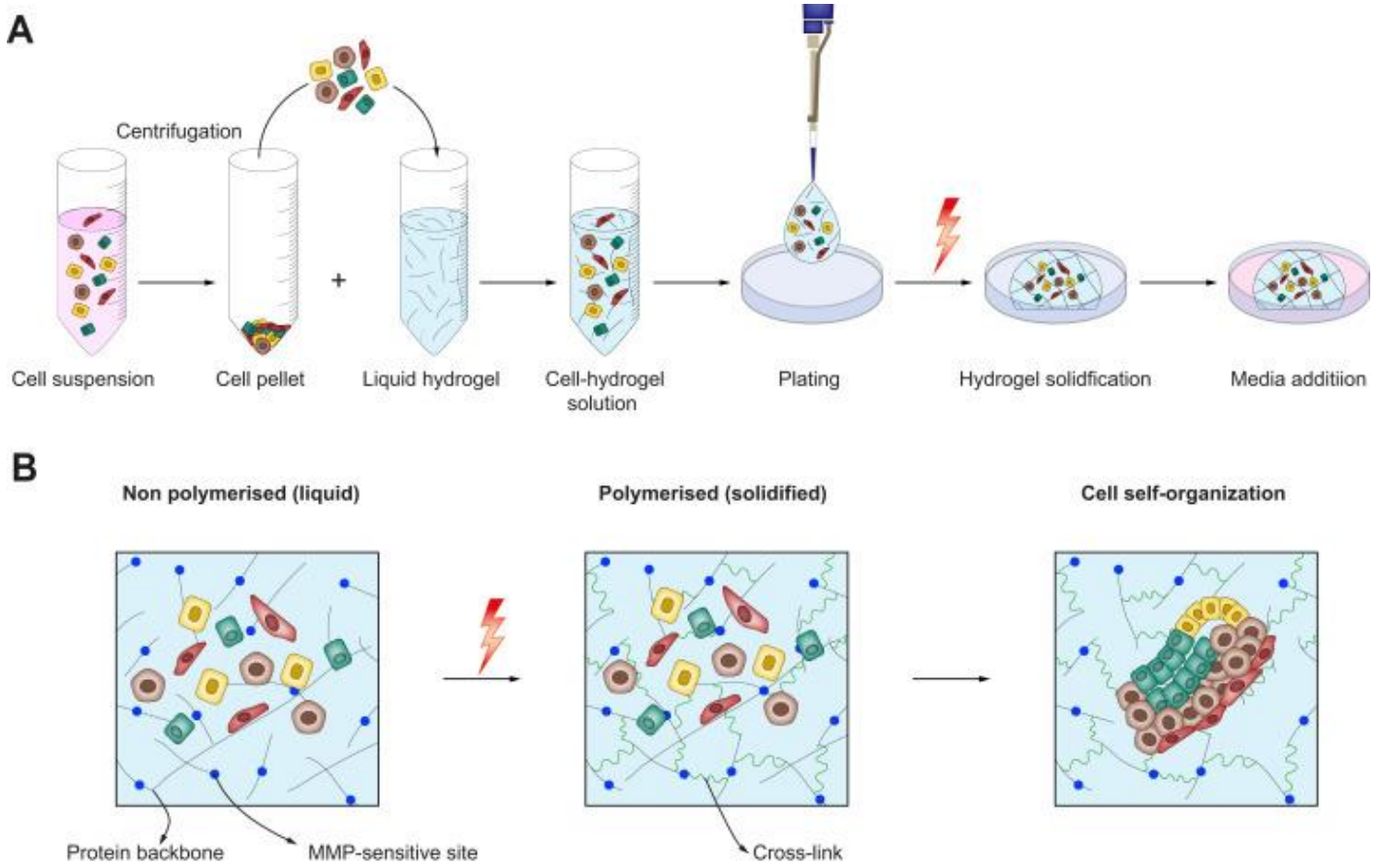
Engineered ECM hydrogel (HepG2) showed excellent engraftment after 3 weeks post implantation. Human positive cells were detected as showed by Ku80 staining (IHC, brown colour). The oementum appears to be the most-optimal implantation site for its vascularization capability

Bio-printed Cholangiocytes: Ductal Formation



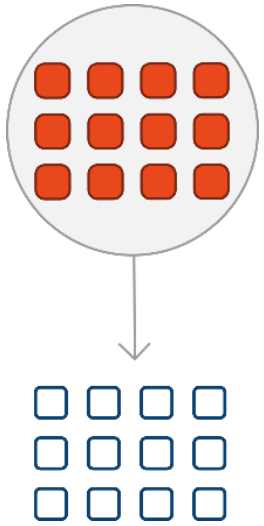
Bile Duct Bio-engineering





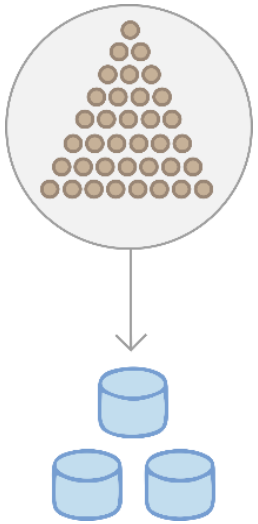
Brevini T et al. J Hepatol 2020;73:918-932

Our Platform Technology: Multiple Applications



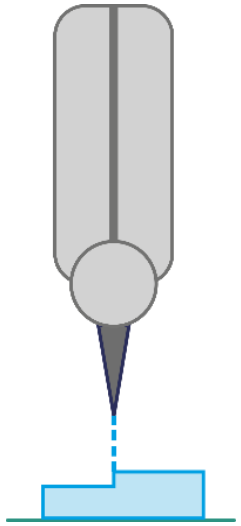
3D-DISEASE MODELLING

- ✓ Modelling of liver fibrosis, metastasis, liver and pancreatic cancer (incl. biomarkers)



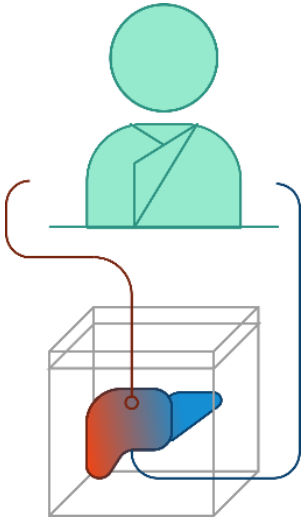
IMPLANTABLE LIVER TISSUE

- ✓ First-in-kind human ECM hydrogel
- ✓ Pre-clinical trials in rodents using iPSC or primary hepatocytes



BIO-INKS FOR 3D BIO-PRINTING

- ✓ First-in-kind human tissue specific

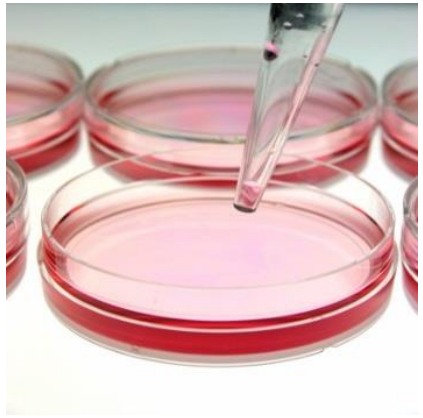


WHOLE ORGAN ENGINEERING

- ✓ First-in-kind human whole liver biological scaffolds
- ✓ R&D recellularization

From Discovery to Clinical Applications

No licensed antifibrotic drugs after more than 40 years of active research (the case of liver fibrosis)



Drug target discovery based on 2D cell cultures on plastic

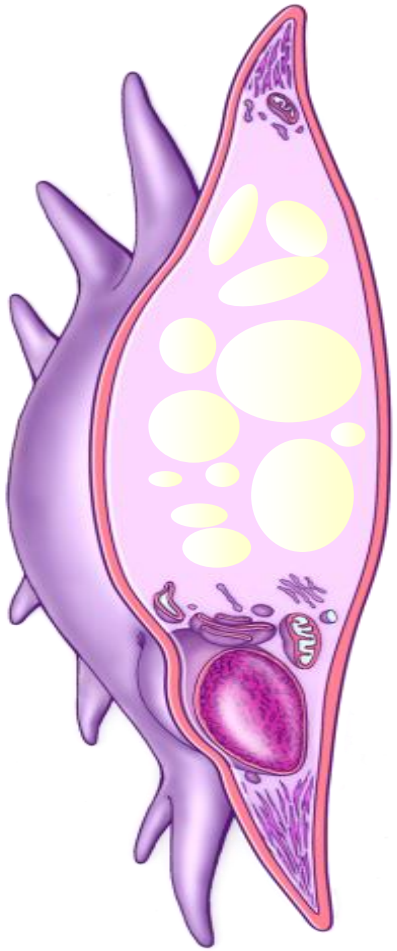
Expansion and validation in animal models of chronic liver injury: no model is able to reproduce human pathophysiology

No translation into clinical trials and very high failure rate in the trials so far performed (>95%)

Wrong targets?

Wrong validation methodology?

Understanding Liver Fibrosis

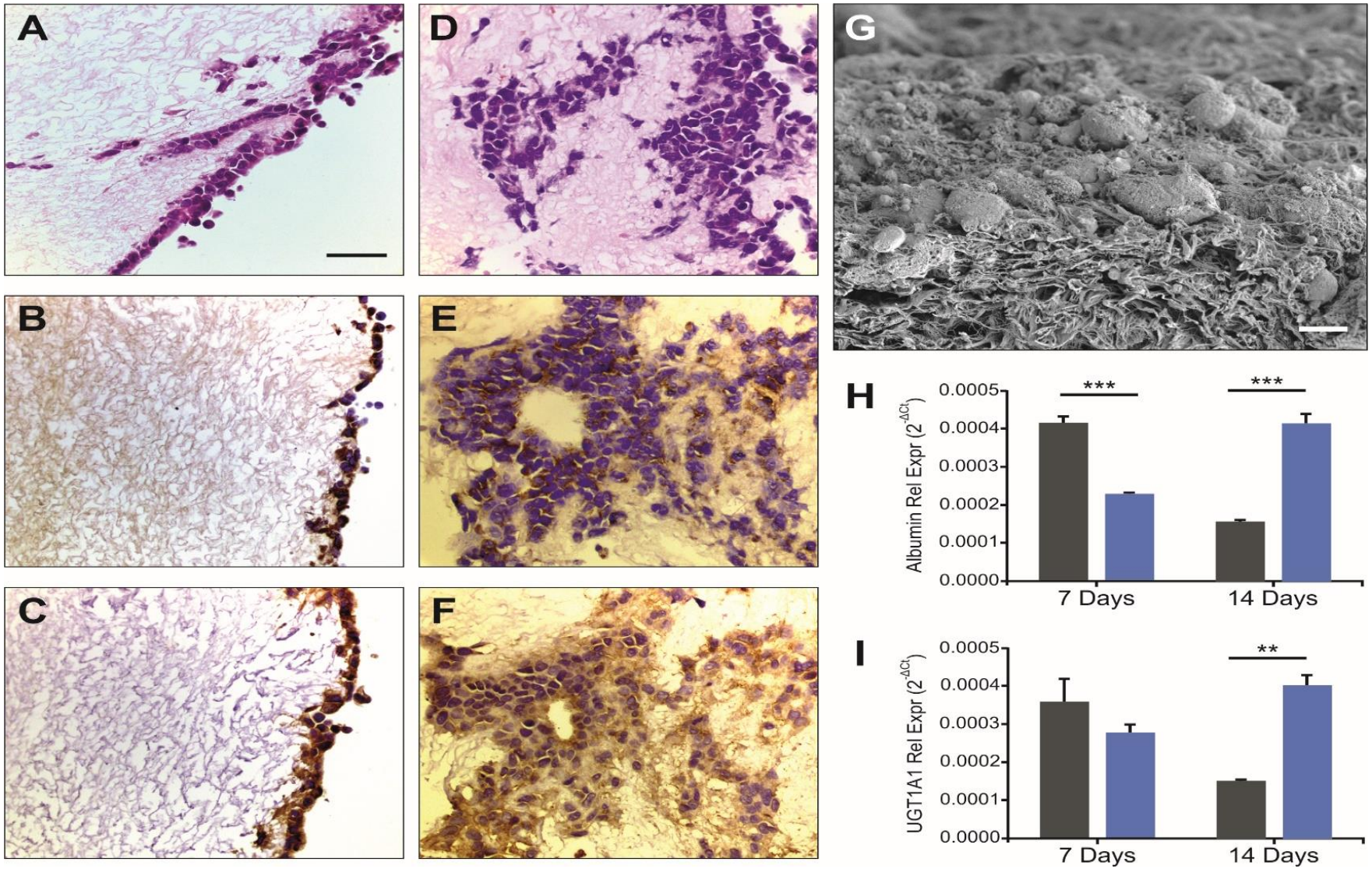


Need to focus on mechanisms and preclinical models easier to translate into clinical applications:

The fibrotic microenvironment :

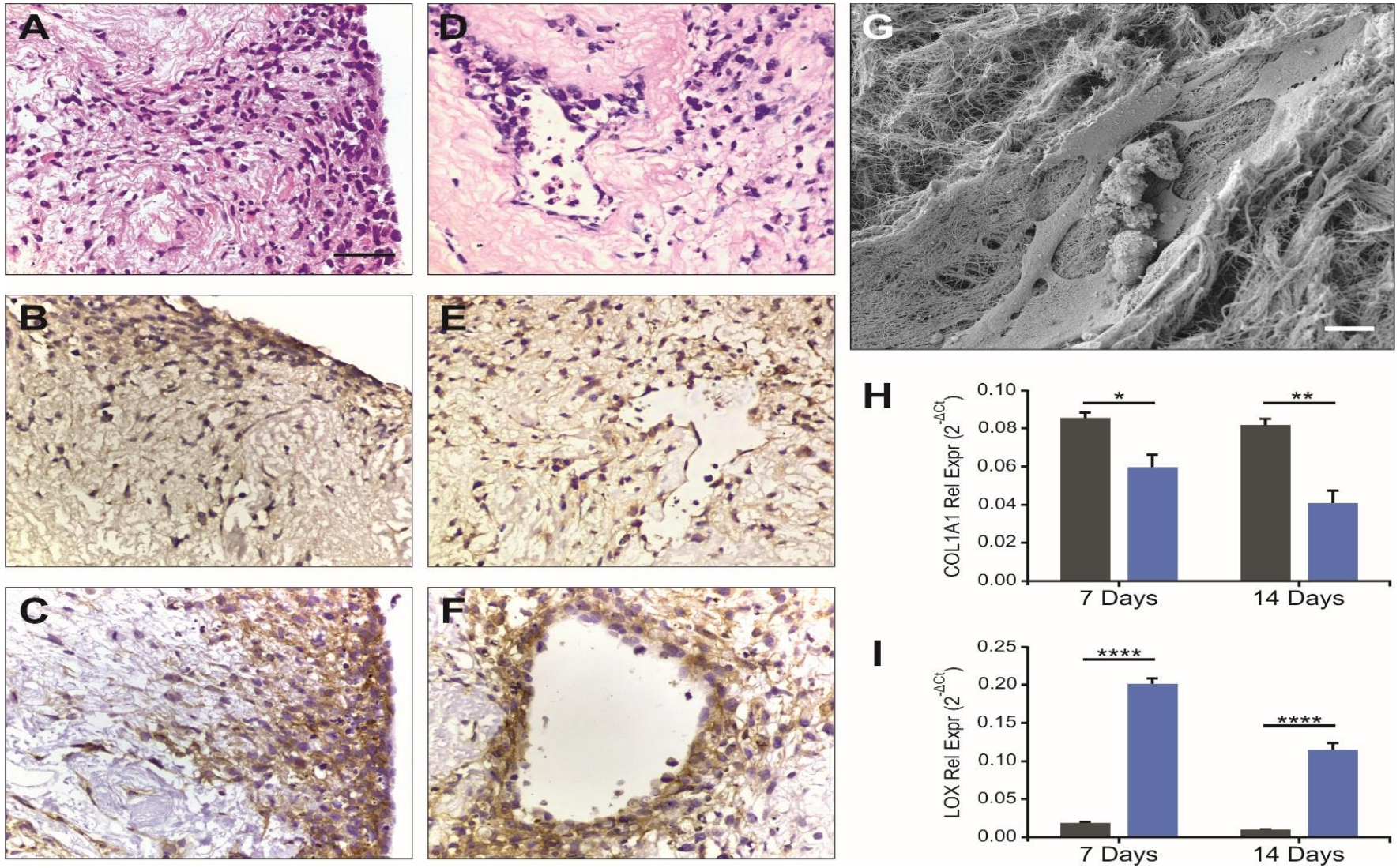
a. Hypoxia and neo-angiogenesis
b. Anaerobic metabolism (e.g. lactate)
c. 3D in vitro models
d. Hepatic matrisome
e. Tissue stiffness and contraction

3D Scaffold Bio-engineering: Hepatocytes



Mazza G *et al.* Sci Rep 2017

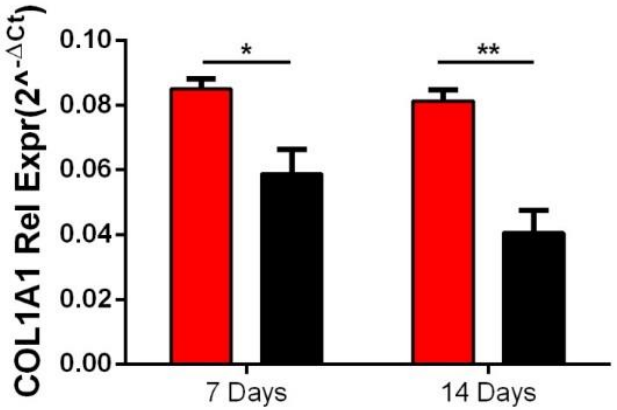
3D Scaffold Bio-engineering: Stellate Cells

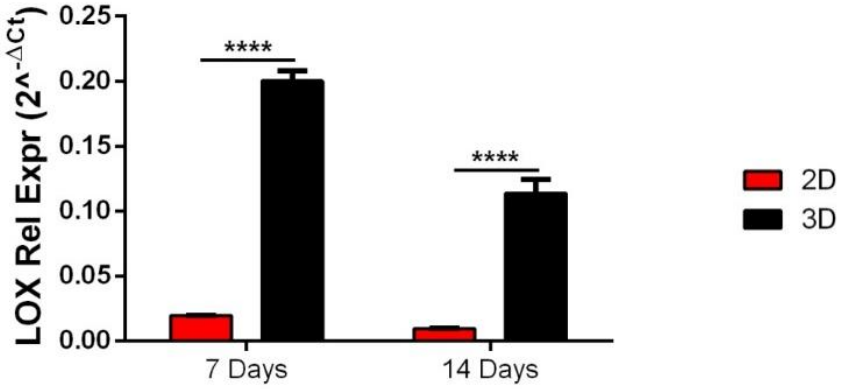


Mazza G *et al.* Sci Rep 2017

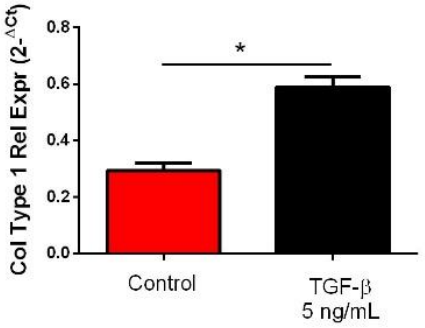
HSC Gene Expression: 2D vs. 3D

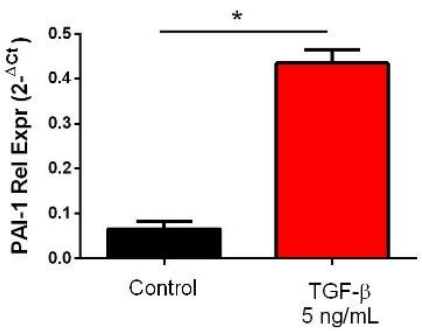
LX2 cells grown in 3D ECM scaffold from healthy liver present a less activated phenotype when compared with the same cells grown on plastic

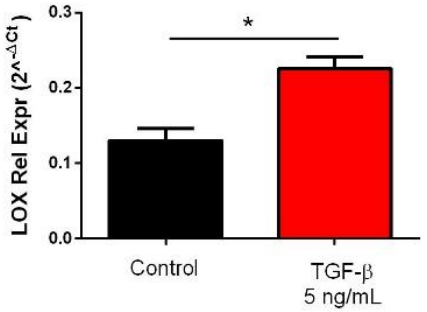


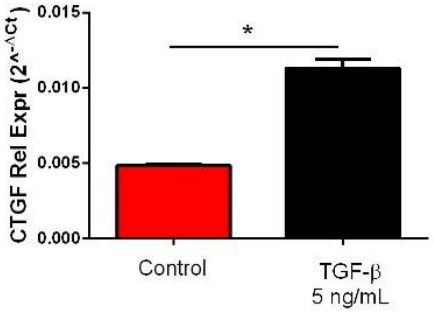


Human liver ECM scaffolds engineered with LX2 stellate cells are highly responsive to TGF-β stimulation and up-regulate key pro-fibrogenic genes









Longato L *et al. EASL NAFLD summit (2017)*

3D Human Scaffold Cultures Vs. Other 3D Systems

Technology/ Criteria	Human Scaffolds	3D Printed (No ECM)	Organoids	Lab-on-a-chip	Liver slices	2D Plastic
Cell Viabilty	●	●	●	●	●	●
Cell Function	●	●	●	●	●	●
Time of Culture	●	●	●	●	●	●
3D Architecture	●	●	●	●	●	●
Tissue-Specific Arch.	●	●	●	●	●	●
Biomechanics	●	●	●	●	●	●
ECM Proteins	●	●	●	●	●	●

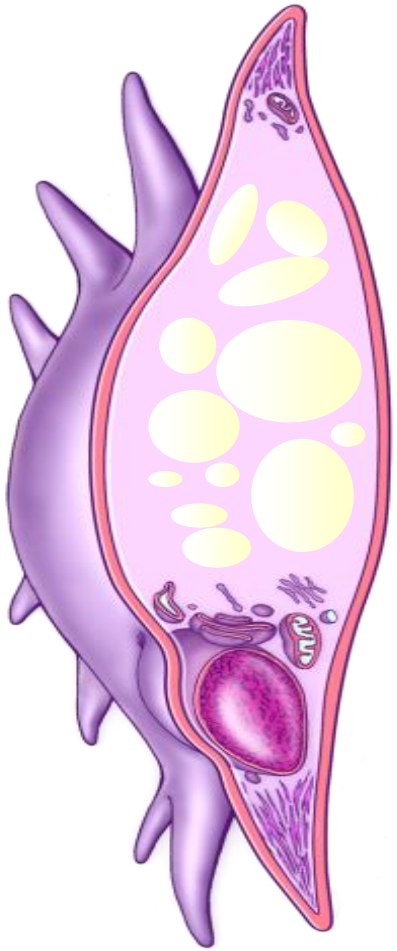
3D Printed in ECM Bio-Inks

● Does not satisfy the criteria

● Does partially satisfy the criteria

● Does satisfy the criteria.

Understanding Liver Fibrosis



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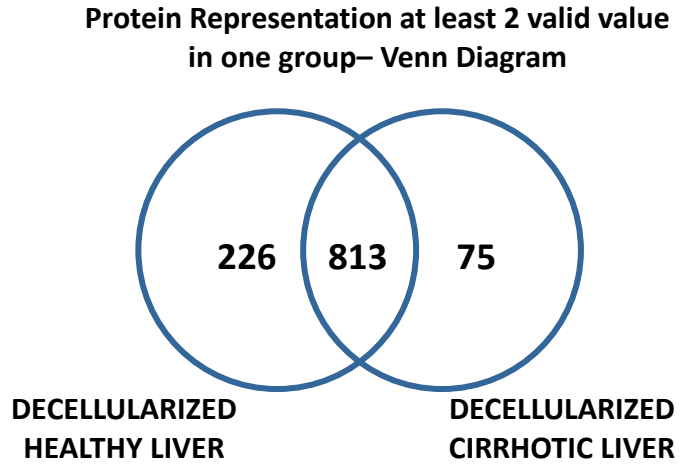
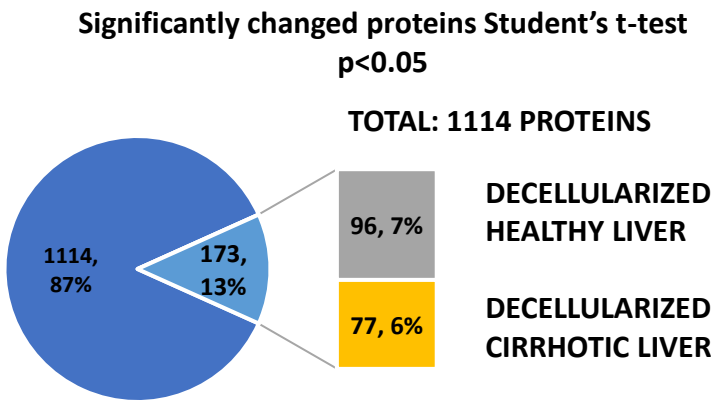
Cirrhotic Human Liver Scaffolds Retain Unique Protein Signatures





Quantitative proteomic analysis of cirrhotic (PSC) and healthy liver scaffolds was performed in order to identify extracellular related proteins. Our previous work (Mazza G et al Sci Rep 2017 and Mangione P, Mazza G et al J Proteomics 2017) firstly described the increase sensitivity of detecting extracellular proteins after decellularization. Quantitative proteomic of cirrhotic and healthy scaffolds identified a total of 1114 proteins. Although cirrhosis is mainly characterized by an increase (quantitative) in already present collagens **is possible to identify unique proteins (quality signatures) in cirrhotic scaffolds with statistical significant change compared to healthy liver scaffolds**

Cirrhotic Human Liver Scaffolds Retain Unique Protein Signatures

Gene names	p-value	Gene names	p-value	Gene names	p-value	Gene names	p-value	Gene names	p-value	Gene names	p-value	Gene names	p-value	Gene names	p-value
IGKV3-11;IGKC	2.13E-05	EFEMP1	0.001654	FBLN5	0.008763	CRTAC1	0.01449	SERPINA1	0.025274	IGKV1D-33	0.030131	IGLC3;IGLC2;IGLC6	0.0394174	HBB	0.044762
BST1	0.000146	HSD17B12	0.002194	CTSS	0.008859	MYO1C	0.01459	H3F3 A-B-C;HIST3H3;	0.026002	UGT2B7	0.030686	NDUFB10	0.039533	LGALS3	0.045656
TMEM43	0.000207	TNS3	0.002660	C1QB	0.009055	RRAS	0.01508	GNAI2	0.026326	IGKV1-5	0.030809	TNS1	0.040605	CXCL12	0.046176
LXN	0.000263	FLOT1	0.004143	S100A9	0.0103688	MYOF	0.01694	PDLIM7	0.026517	APOH	0.031237	GNB1	0.040771	AOC3	0.046702
TPSAB1;TPSB2	0.000441	CLTC	0.004354	LOXL1	0.0105657	FLOT2	0.017647	THSD4	0.026729	FLNA	0.034109	MYO1D	0.041279	COL5A1	0.047362
CMA1	0.000650	IGKV3-15;3-7; IGKV3OR2-268;	0.004652	TGFB1I1	0.0114113	ADH1B	0.018949	IGKV3	0.027050	DRG1	0.034959	SEC61A1;SEC61A2	0.041344	TBL2	0.048181
CPA3	0.000791	IGHG1	0.005110	HLA-DRA	0.011745	IGFBP7	0.019384	HNRNPM	0.028411	EMILIN1	0.036259	FBLN2	0.041856	IGHG3	0.049672
DNAJB9	0.000965	CTSG	0.005589	TRAM1	0.0121993	FBLN1	0.019681	FBN1	0.028437	PSMA5	0.036515	IFI16	0.042079		
FHL2	0.001114	LAMA2	0.005909	GPX3	0.0130434	VCAN	0.020773	EHD2	0.029022	ADH1A	0.037810	ARPC4-TTLL3;ARPC4	0.042175		
IGKV2D-28	0.001222	MFAP4	0.006719	SEC22B	0.0131714	TRIM25	0.022131	LTBP1	0.029929	VDAC2	0.039389	CAV1	0.044028		

Unique protein signatures showed key proteins (gene names) involved in fibrosis/cancer of other organs or already described in liver diseases. Key proteins (highlighted in red) are currently under investigation for biomarkers and/or therapeutics development.

INNOVATION & ENTERPRISE

UCL

Awards for Innovation and Enterprise 2019

Dr Giuseppe Mazza

UCL Provost's Spirit of Enterprise Award

Prof. Krista Rombouts

UCL Institute for Liver and Digestive Health