iPSC-derived liver spheroids for liver disease and toxicity assessment

Final Symposium
Brussels
November 3, 2021

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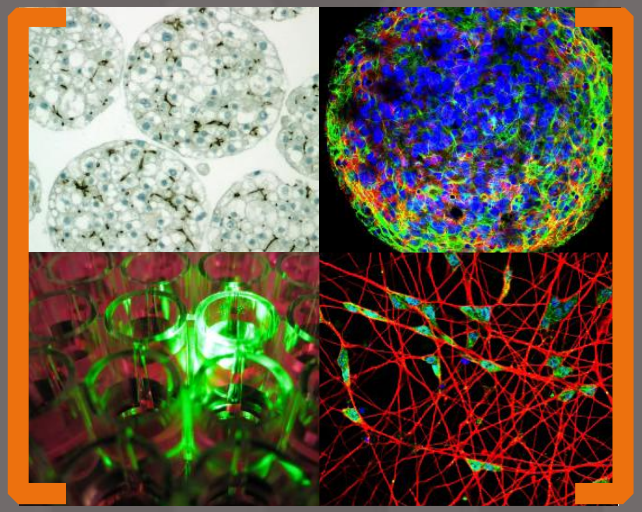
KU Leuven
Belgium

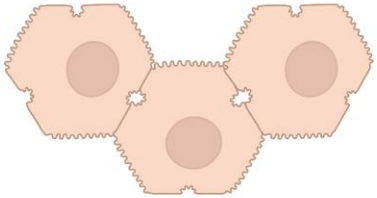


The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

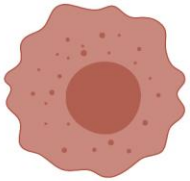
Current liver models used in liver toxicology and disease modeling

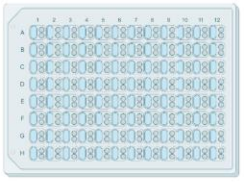
rodent models

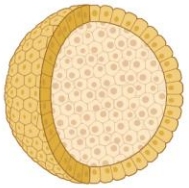
PHHs



HepG2, HepaRG







2D culture --> Sandwich/Spheroid

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Pluripotent stem cell derived models: why?

- Steady source of long term expandable stem cells
- Can be derived from individuals with different disease/toxicity susceptibility
- Can generate (in theory) all cells from the human body
- Can be relatively easily genome edited, e.g. to build in stress reporters, …

Pluripotent stem cell derived models: why not yet?

- Differentiation process does not yet create fully mature primary organ like cells
- Need to be validated vs. established models
- Require regulatory acceptance

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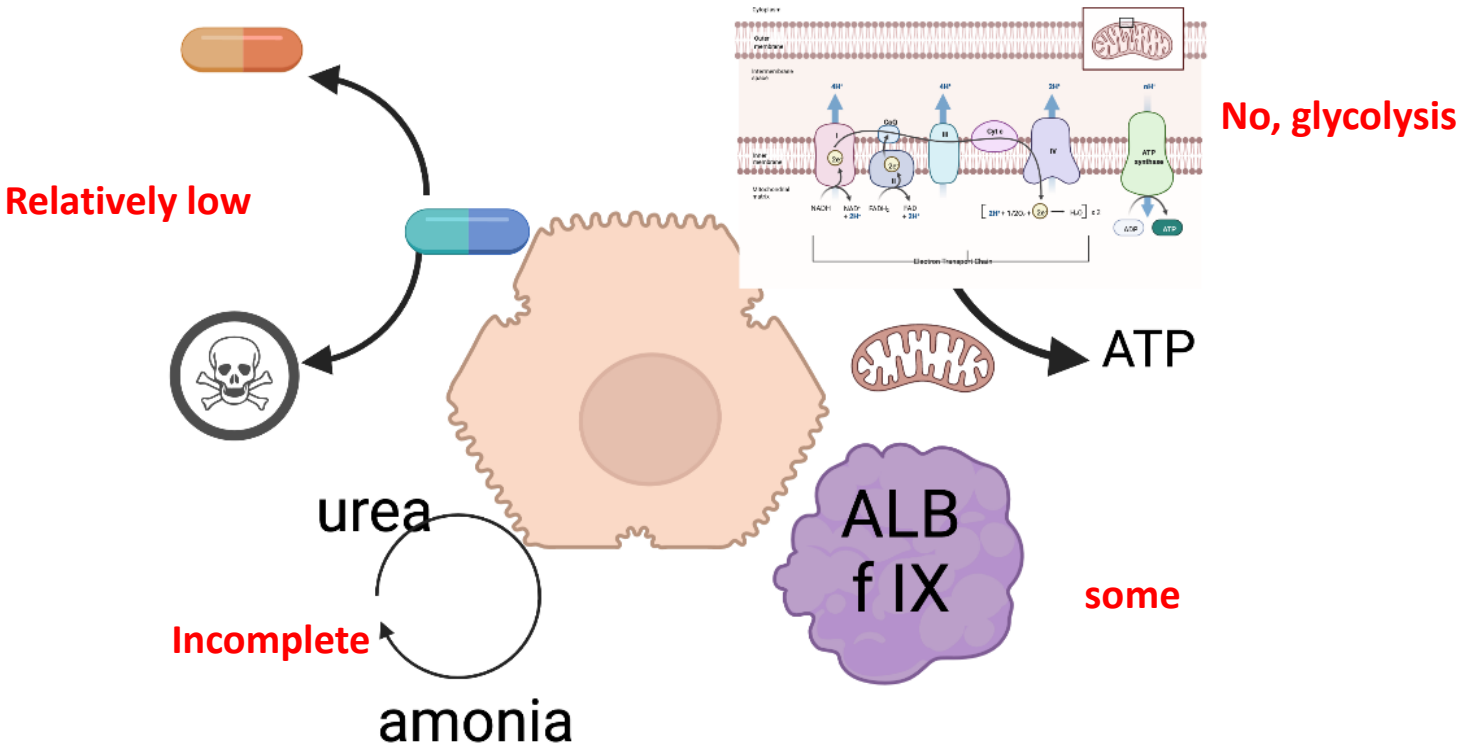
Pluripotent stem cell derived hepatocytes



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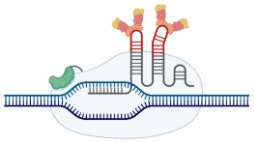
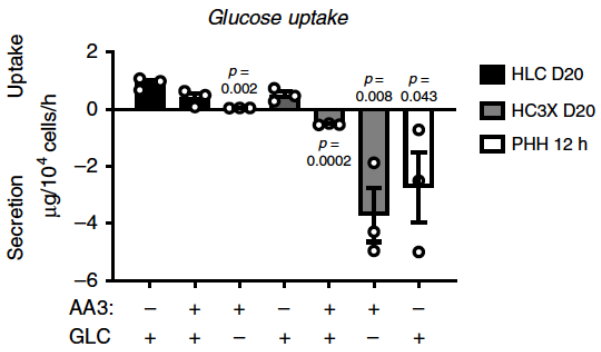
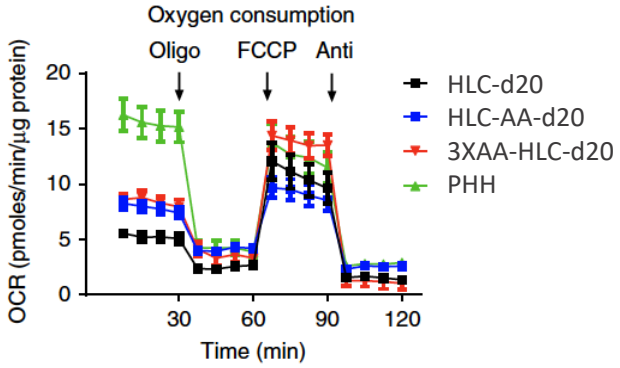
3XAAGly hepatocyte like cells (HLCs) derived from PSCs have mature cell metabolism and significantly improved drug biotransformation/ sensitivity

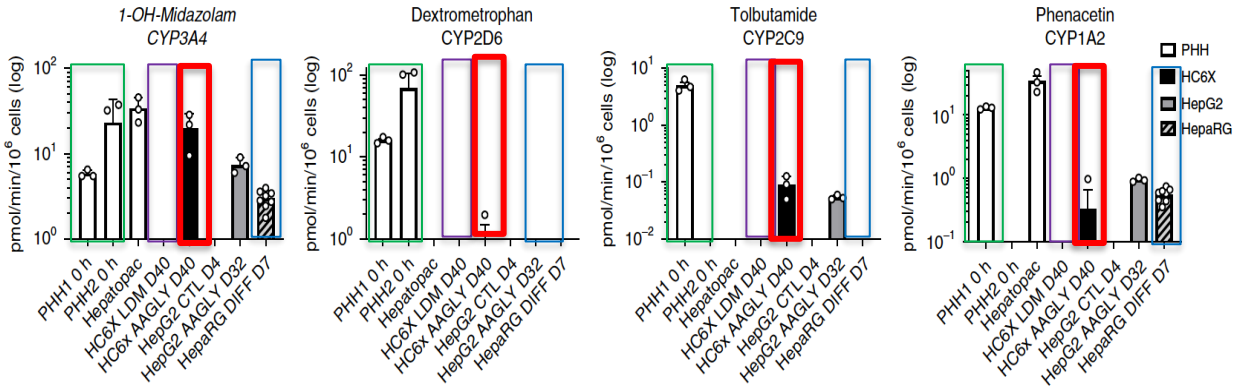


TF drived differentiation
(HNF1, PROX1, FOXA3)
=3X

= AAGly
Metabolic engineering
(Very high concentration AAs)







Boon et al, Nature Communications, 2020

EUTOXRISK

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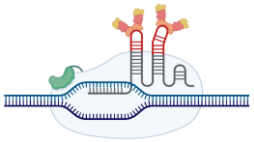
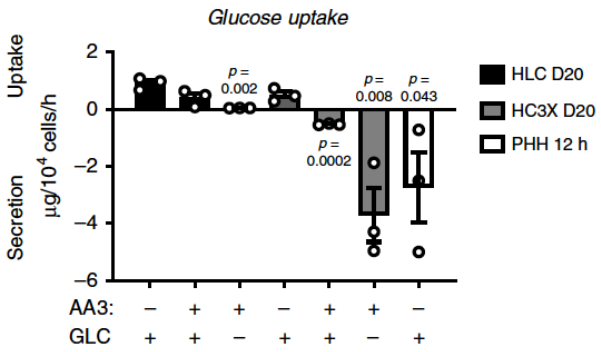
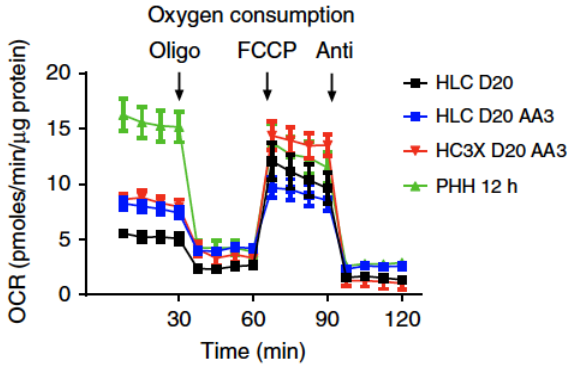
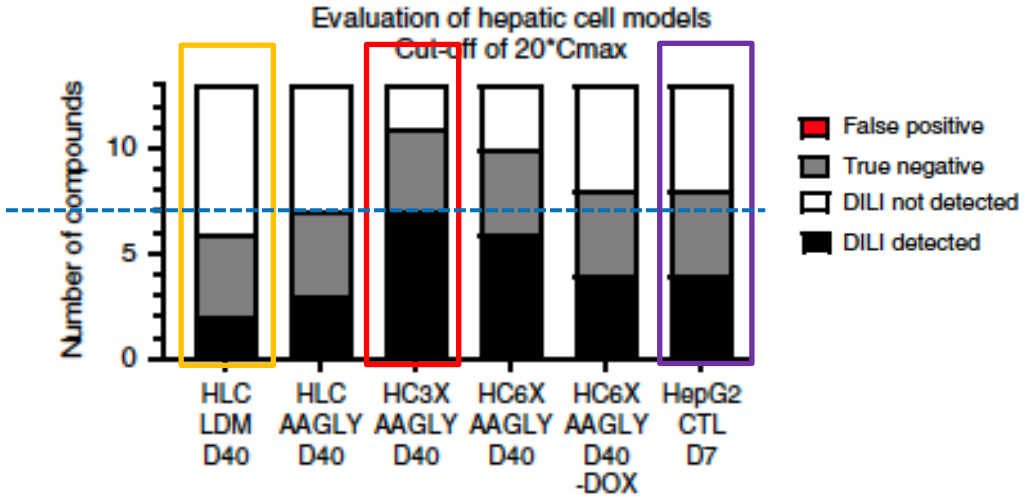


TF drived differentiation
(HNF1, PROX1, FOXA2)
=3X

= AG
Meatbolic engineering
(Massive increase in AA)







Boon et al, Nature Communications, 2020

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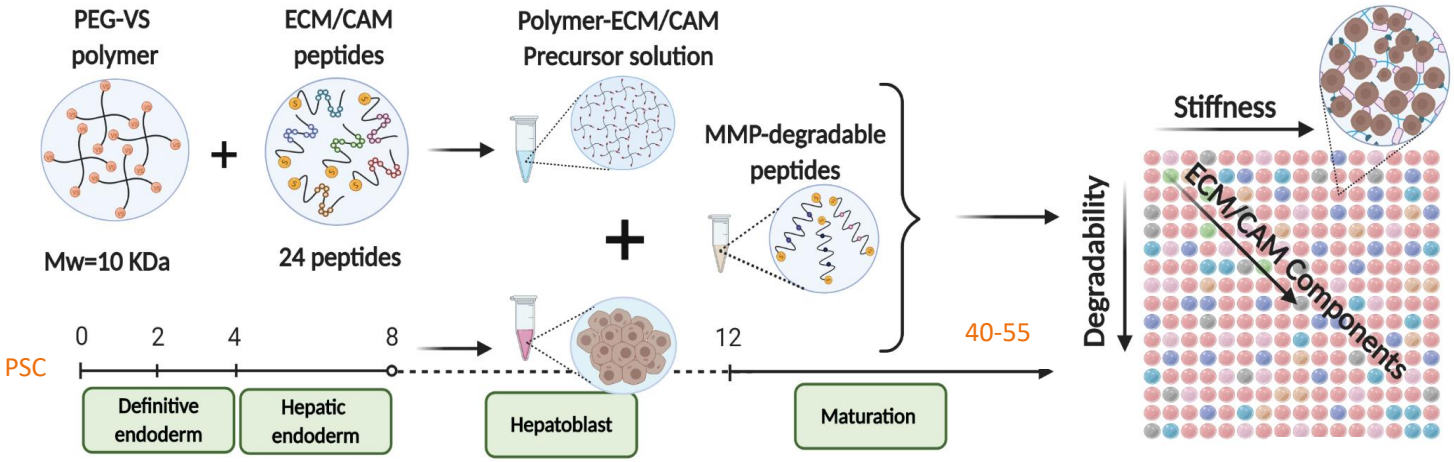
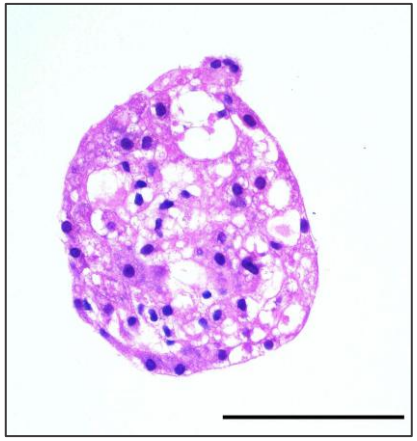
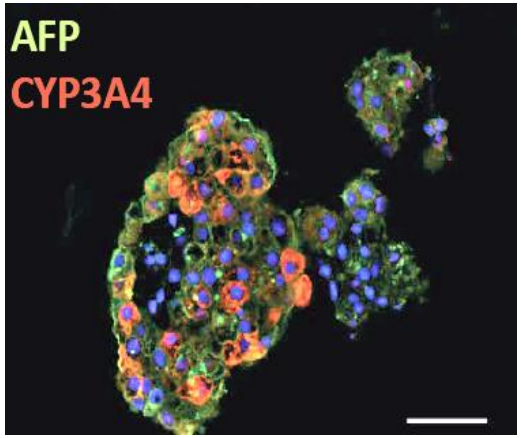
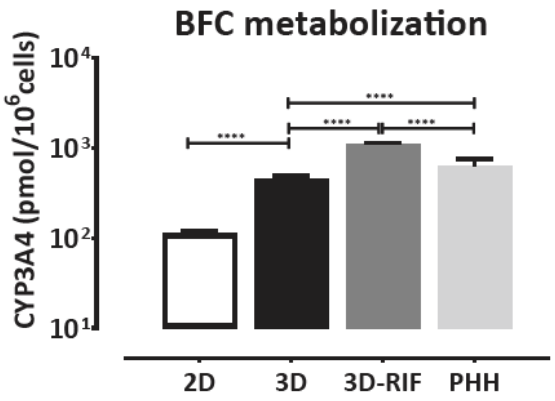
3XAaGly-HLCs cultured in 3D functionalised PEG hydrogels: improved functionality









Kumar, Biomaterials 2021

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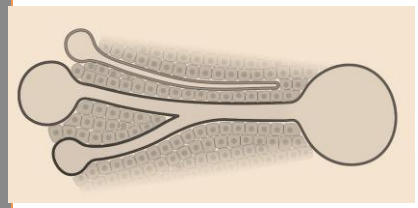
Liver more than hepatocytes: PSC-derived non-parenchymal cells (NPCs)

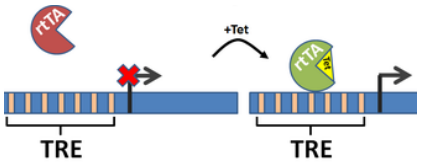


hepatocyte

LSEC

HSC

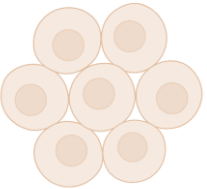
KC/Mϕ



ETV2
SPI1

Growth factors / Cytokines / Palmitate

EBs + Growth factors / Cytokines



Desmedt , Cell Death & Diff, 2021 ; Col, Cell Stem Cell 2018;
Vallverdú, Nat Protocols, 2021; Claes , Alz&Dem, 2019

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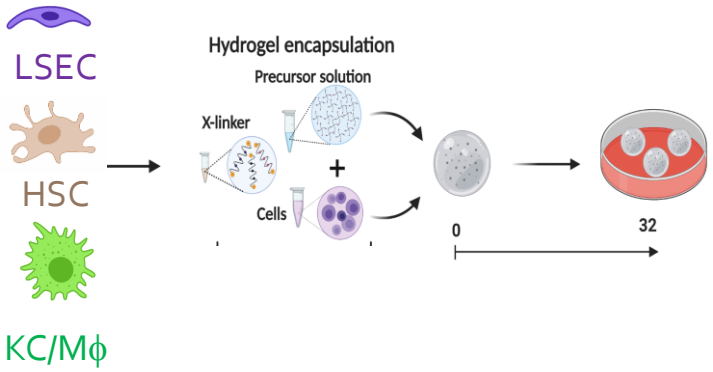
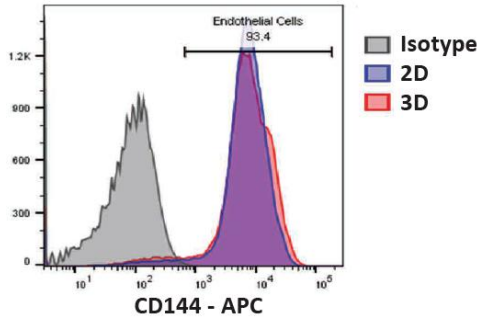
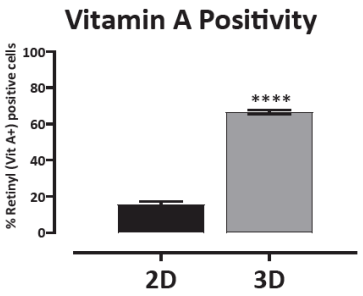
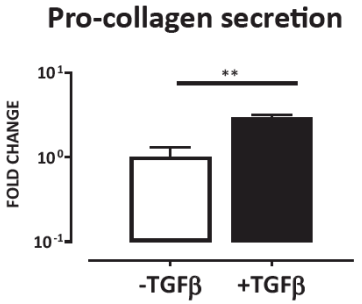
EUTOXRISK

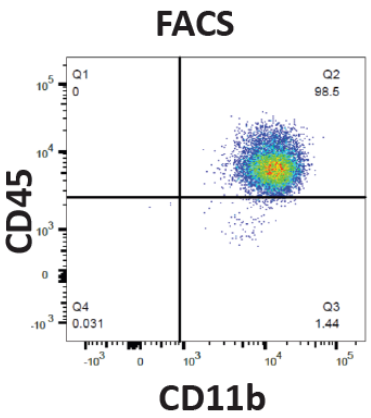
PSC-NPCs can be maintained functional for =/> 32 days in HepMat hydrogel

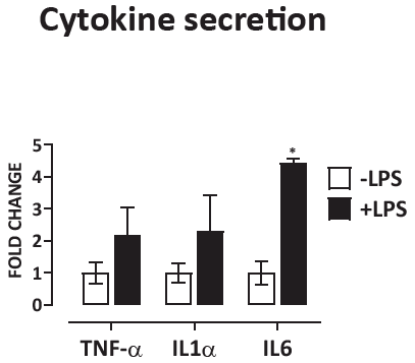












Desmedt , Cell Death & Diff, 2021 ; Col, Cell Stem Cell 2018; Vallverdú, Nat Protocols, 2021; Claes , Alz&Dem, 2019

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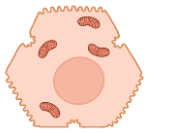
3XAAGly HLCs and PSC-NPCs can be cocultured long term in 3D functionalised HepMat hydrogels



hepatocyte

LSEC

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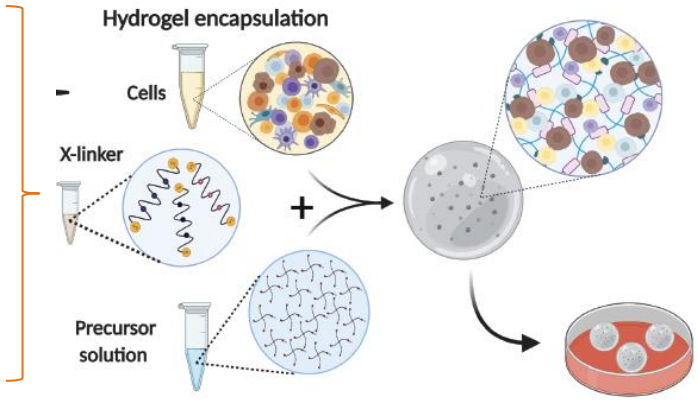
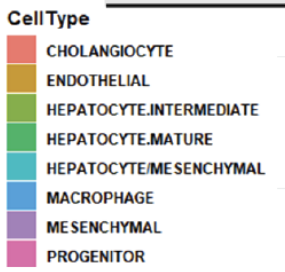


Cell Type	Frequency	Percentage	Abs used
Cholangiocyte	913	4.68	KRT19
Endothelial	381	1.95	CD31
Macrophage	2455	12.57	CD68
Mesenchymal	2633	13.48	VIM
Hepatocyte Intermediate	895	4.58	HNF4a, PEPCK
Hepatocyte Mature	3051	15.62	CYP3A4, ALB
Hepatocyte Mesenchymal	1249	6.39	VIM, ALB
Progenitor	1265	6.48	AFP, KRT19
Blank/Other	6692	34.26	No Signal present



Kumar, Biomaterials 2021

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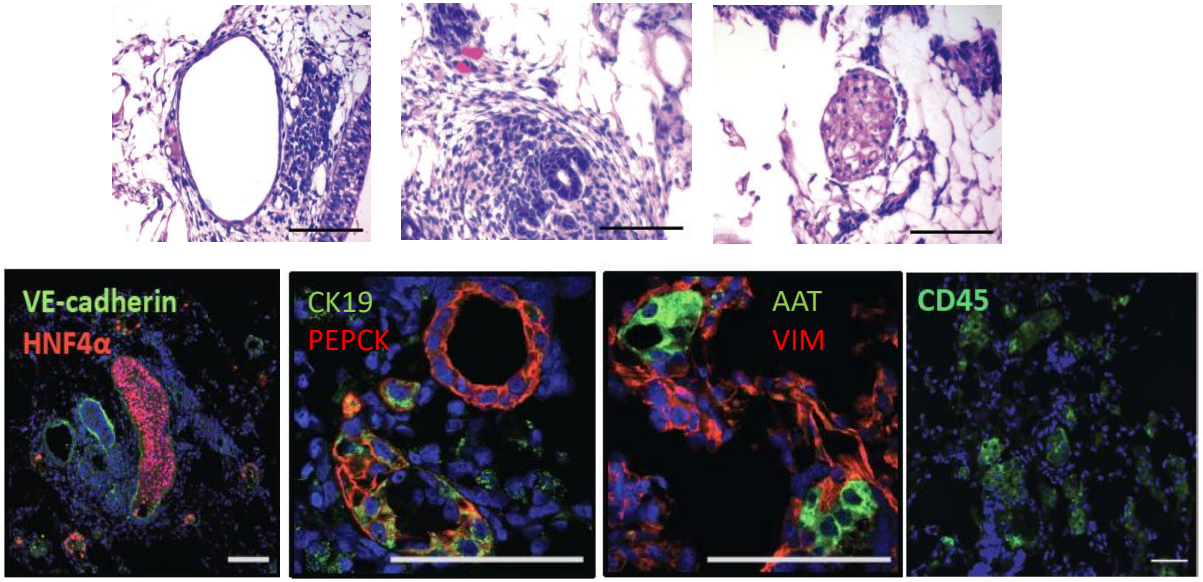

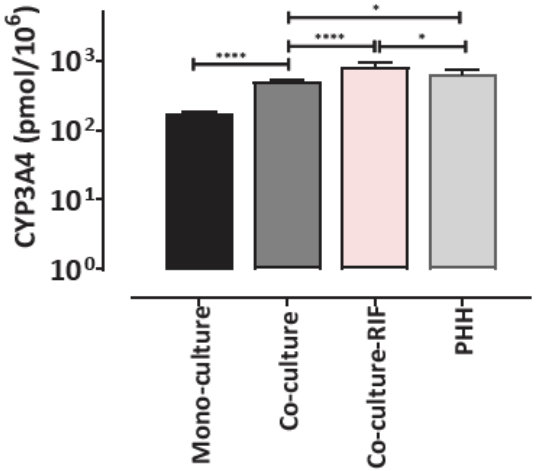

Kumar, Biomaterials 2021

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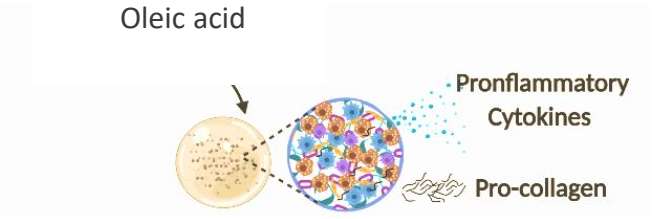
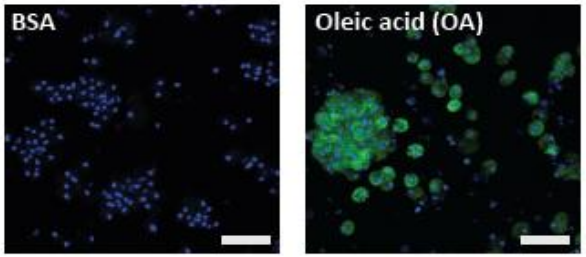
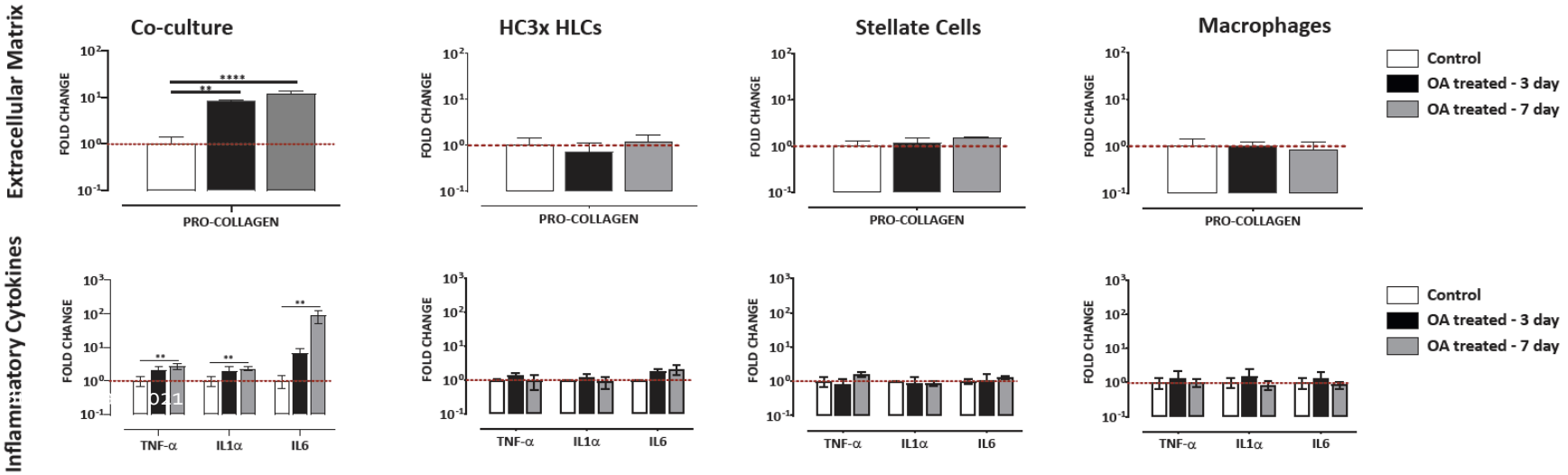
Lipid induced fibrosis and inflammation only detected by coculture of 3XAAGly HLCs and PSC-NPCs

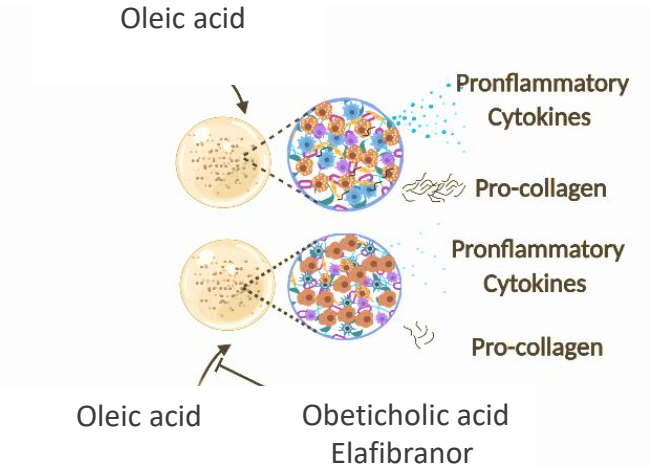


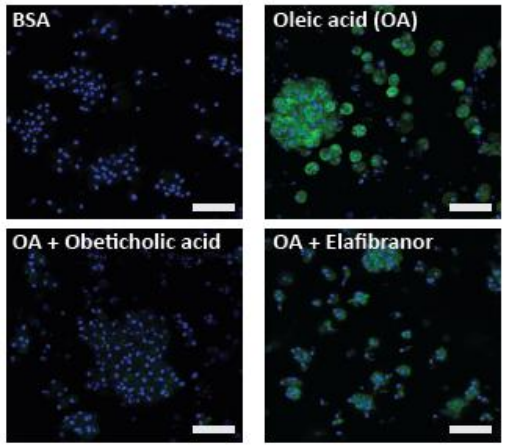


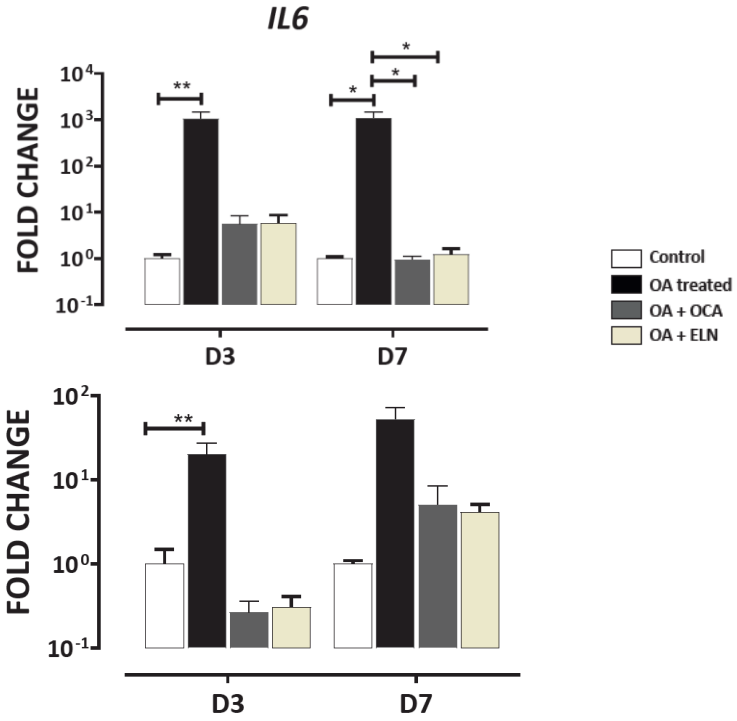


Kumar, Biomaterials 2021

Drugs decreasing fibrosis and inflammation may be identified in cocultures of 3XAAGly HLCs and PSC-NPCs







Kumar, Biomaterials 2021

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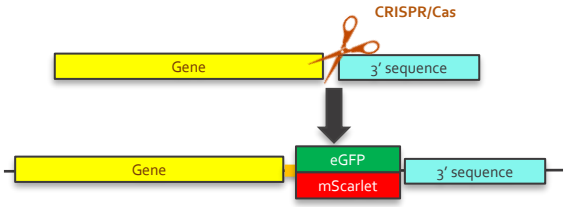
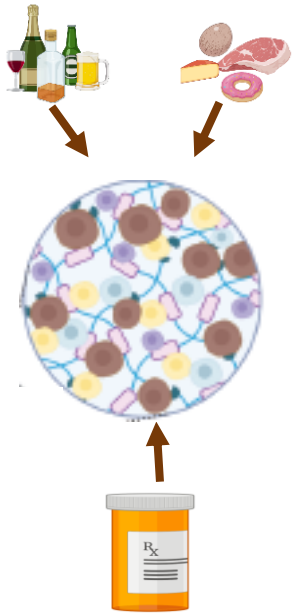
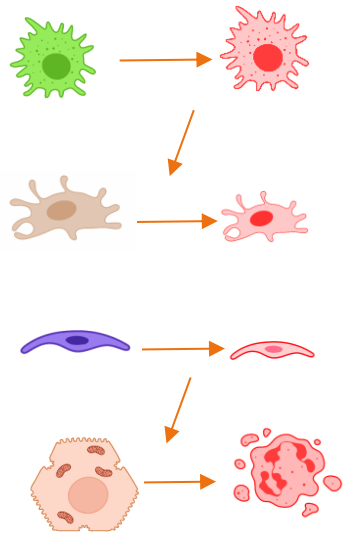
Use of reporter iPSC lines for mechanistic toxicology/disease modeling

	Oxidative stress	ER stress		Inflammatory stress
sensor	KEAP1			TNFR
TF	NRF2	XBP1	ATF4	NFκB
target	SRNX1	BIP	CHOP	ICAM1
	…	…	…	…





Possible Mechanism:



Snijders et al, Arch Toxicol 2021

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Conclusions

Genomic and metabolic engineering enhances PSC-derived hepatocyte (like cell) function

Cells with non-parenchymal cell features can be generated from PSCs

3XAAGly Heps maintained for >1 month in HepMat hydrogels have improved functionality

The HepMat hydrogel also supports non-parenchymal cells for > 40 days

3XAAGly HLCs + non-parenchymal cell coculture in HepMat hydrogels supports all four cells, and further improves 3xAG maturation

The HepMat coculture system allows NASH modeling

Studies ongoing to assess susceptibility of HepMat cocultures to DILI and iDILI drugs

Constructs with stress reporters may aid in mechanistic disease modeling and toxicity assessment

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Acknowledgements

KU LEUVEN

Current members

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Rodrigo Furtado
Mostafa Kiamehr
Gert Van Marcke
Tine Tricot
Jonathan De Smedt
Sreya Gosh
Niels Vidal
Rob Van Rossem

Past members

Laura Ordovas
Ruben Boon

Many many more

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Adrian Ranga (KUL)
Tania Roskams (KUL)
Yvan Saeys (VIB Gent)
Pau Sancho (IDIBAPS)
Charlotte Scott (VIB Gent)
Leo van Grunsven (VUB)

IWT-SBO-HILIM-3D team
FP7-HeMiBio team
H2020-EU-ToxRisk team

fwo
iwt
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Galápagos
RISK HUNT3R

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