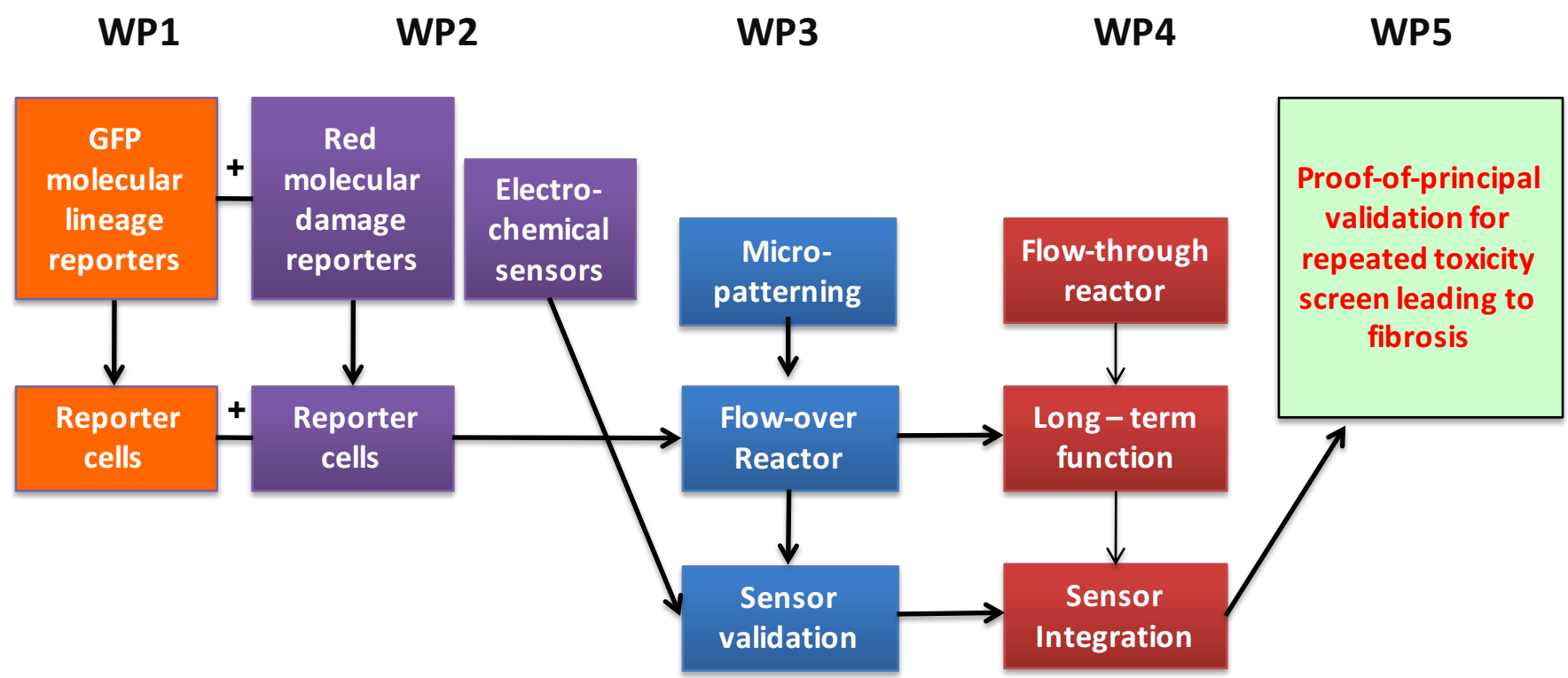
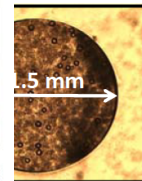
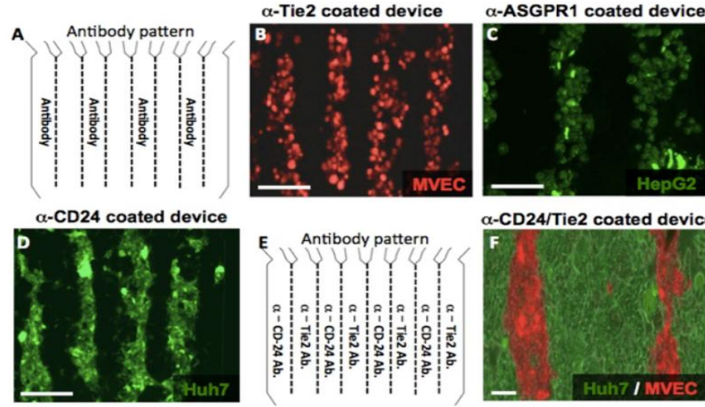
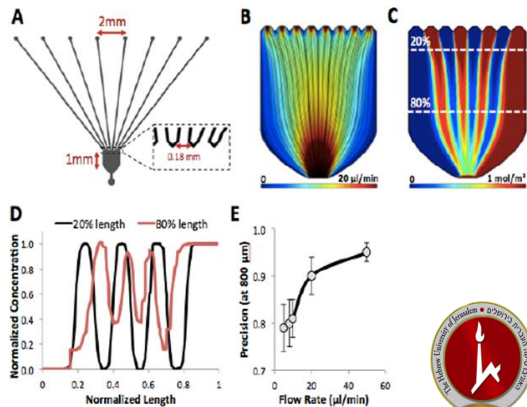


HeMiBio objective: to create in vitro culture system to evaluate liver fibrosis





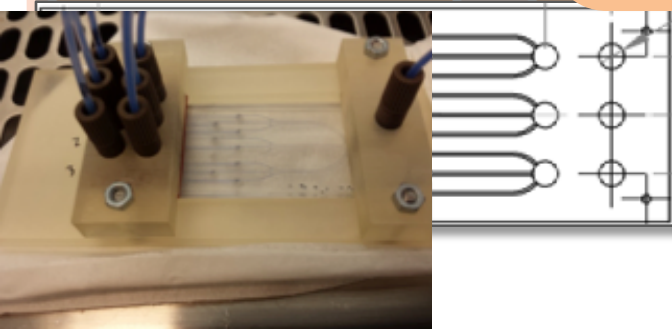
BIOREACTORS

- Micropatterned
- Flow over
- Flow through

SENSORS

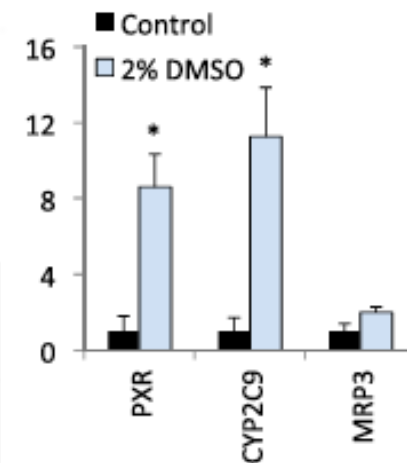
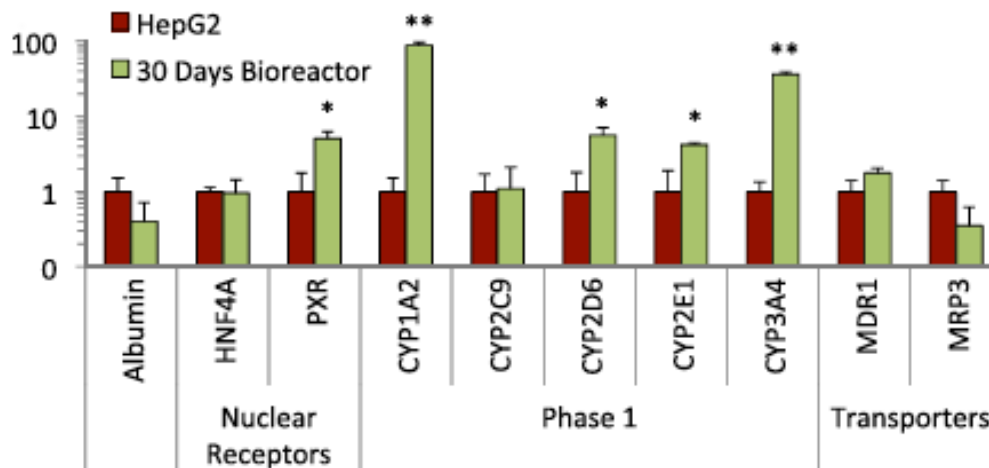
CELLS

Fibrosis



Cosmetics Europe
the personal care association

Long-term maintenance of HepG2 cells in flow over 2D bioreactor



BIOREACTORS

- Micropatterned
- Flow over
- Flow through

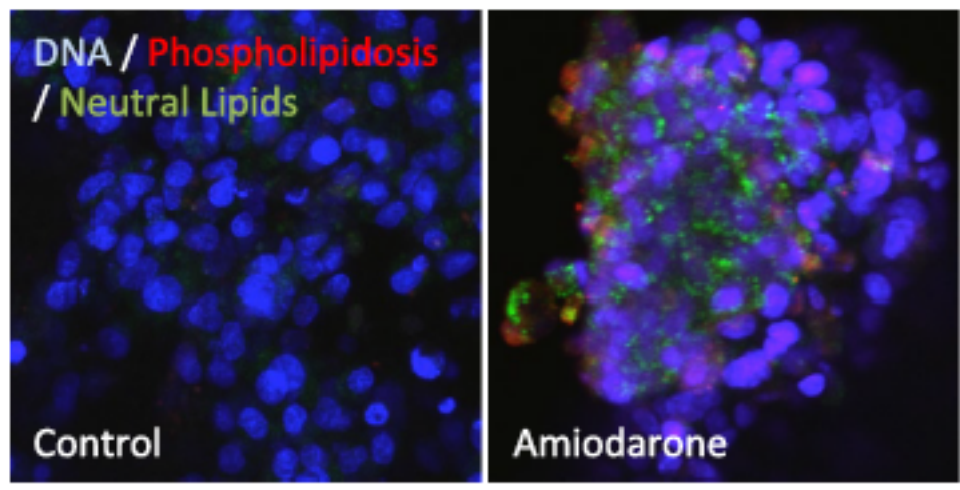
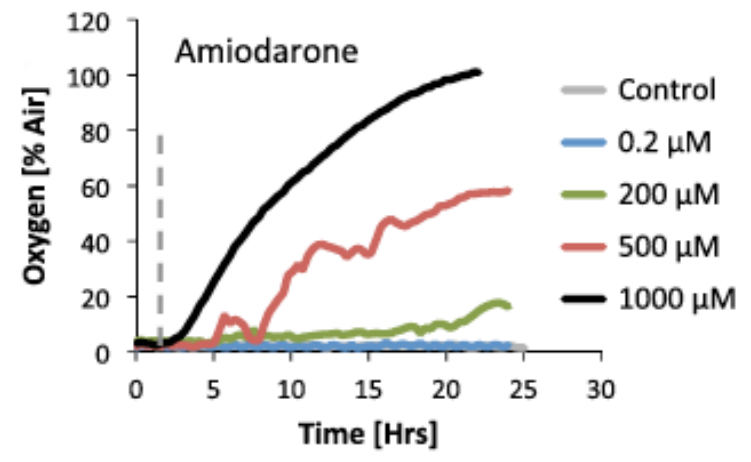
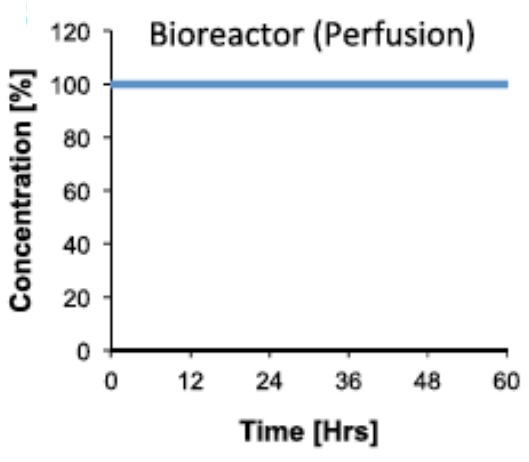
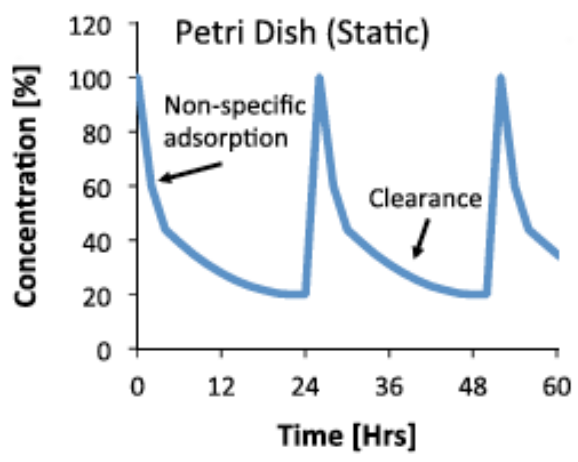
SENSORS

Fibrosis

- Extracellular sensors
 - Frequency based phosphorescence oxygen sensor
 - Electrodes: glucose, lactate, urea, glutamate
- Intracellular sensors

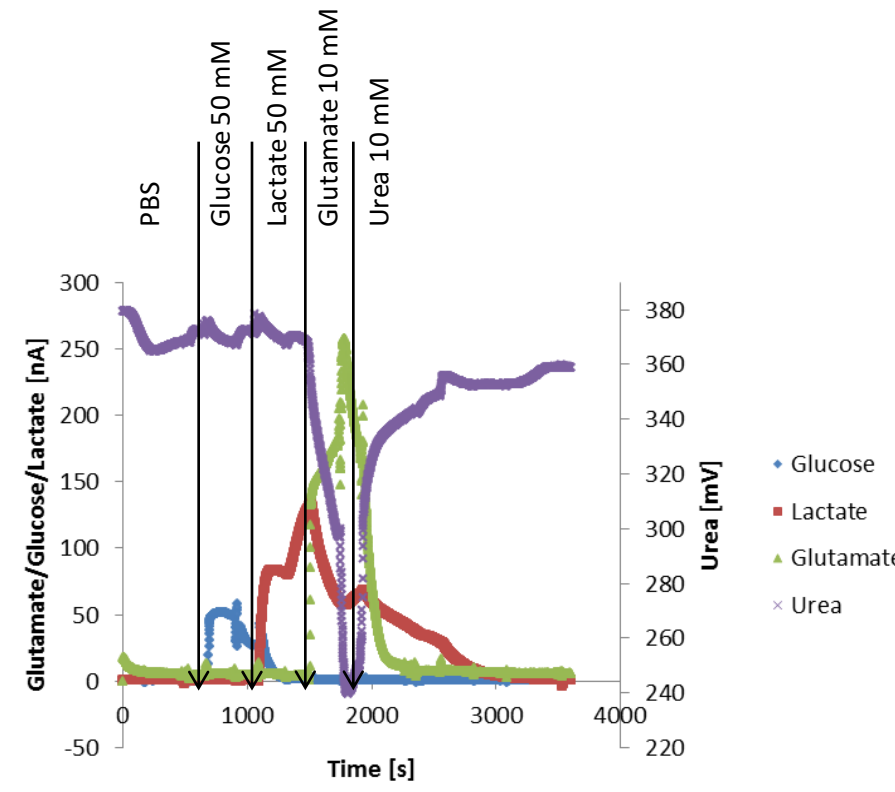
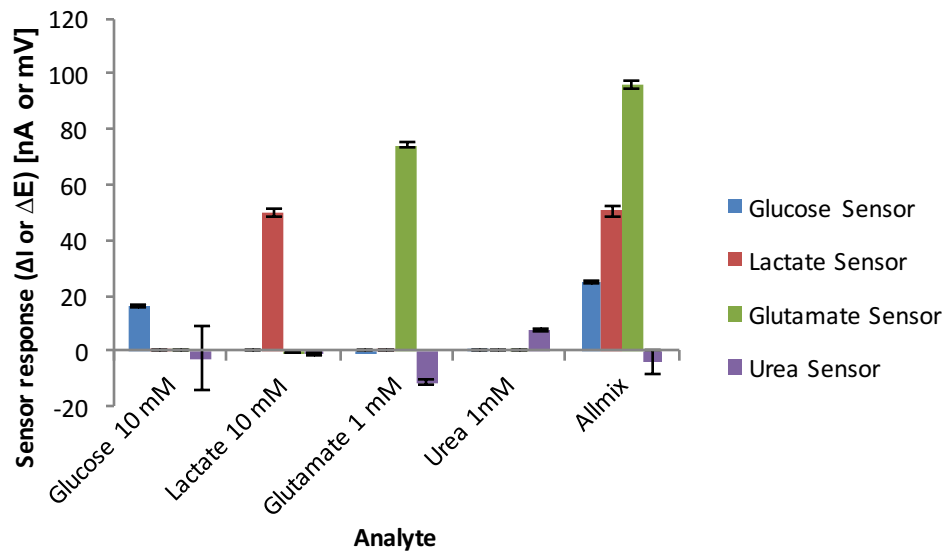
On-chip (2D flow over bioreactor) toxicity measurement based on a frequency-based phosphorescence oxygen

Bioreactor

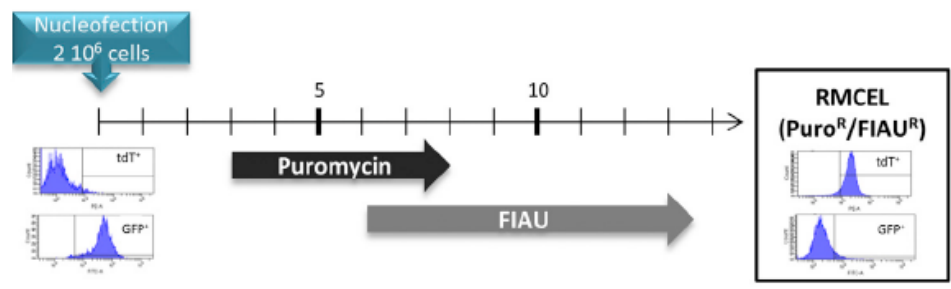
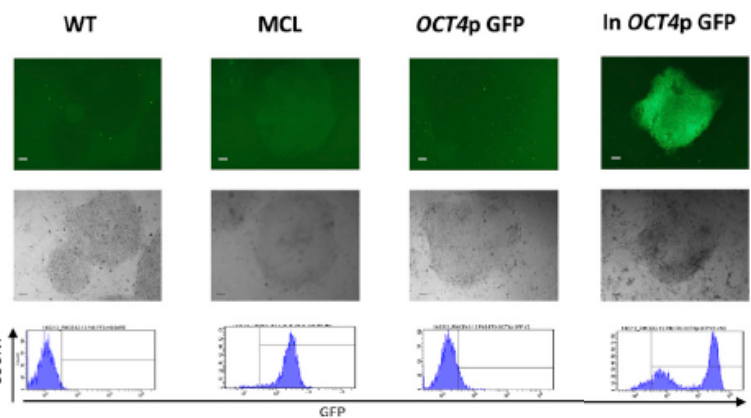
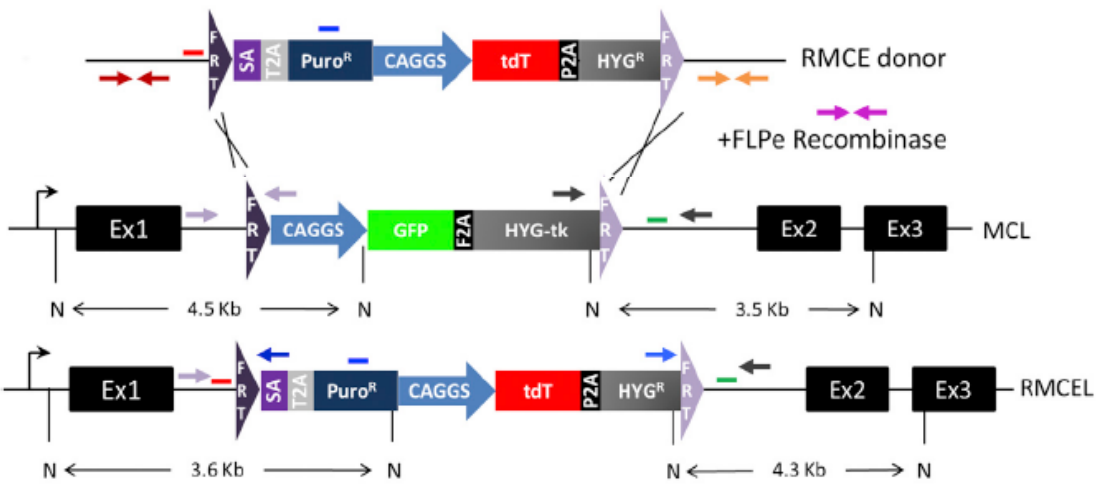


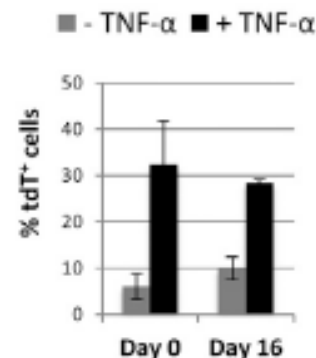
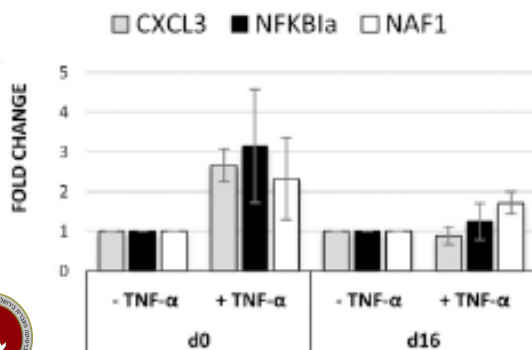
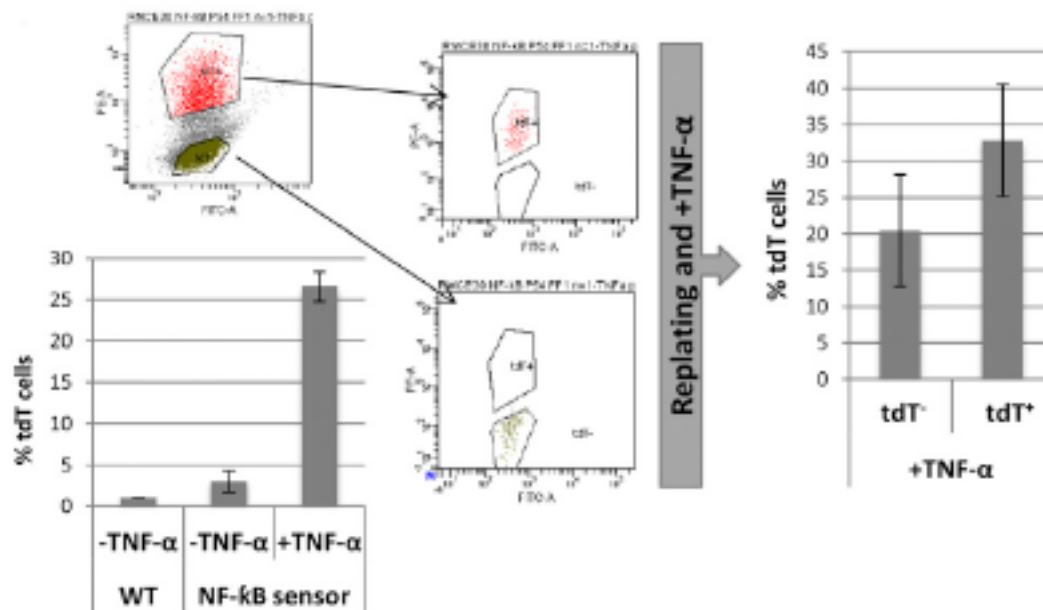
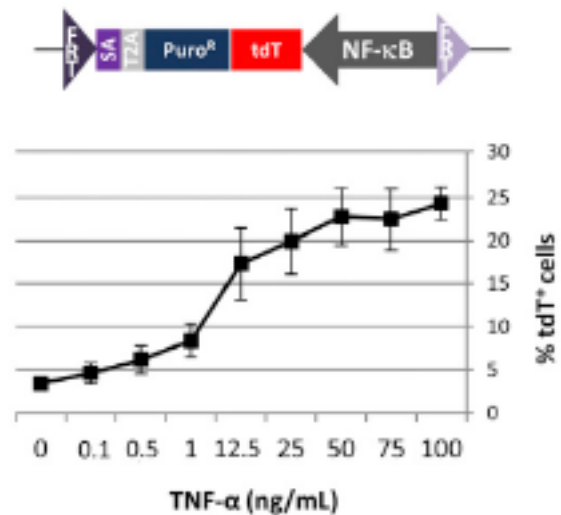
Interference/crosstalk measurements

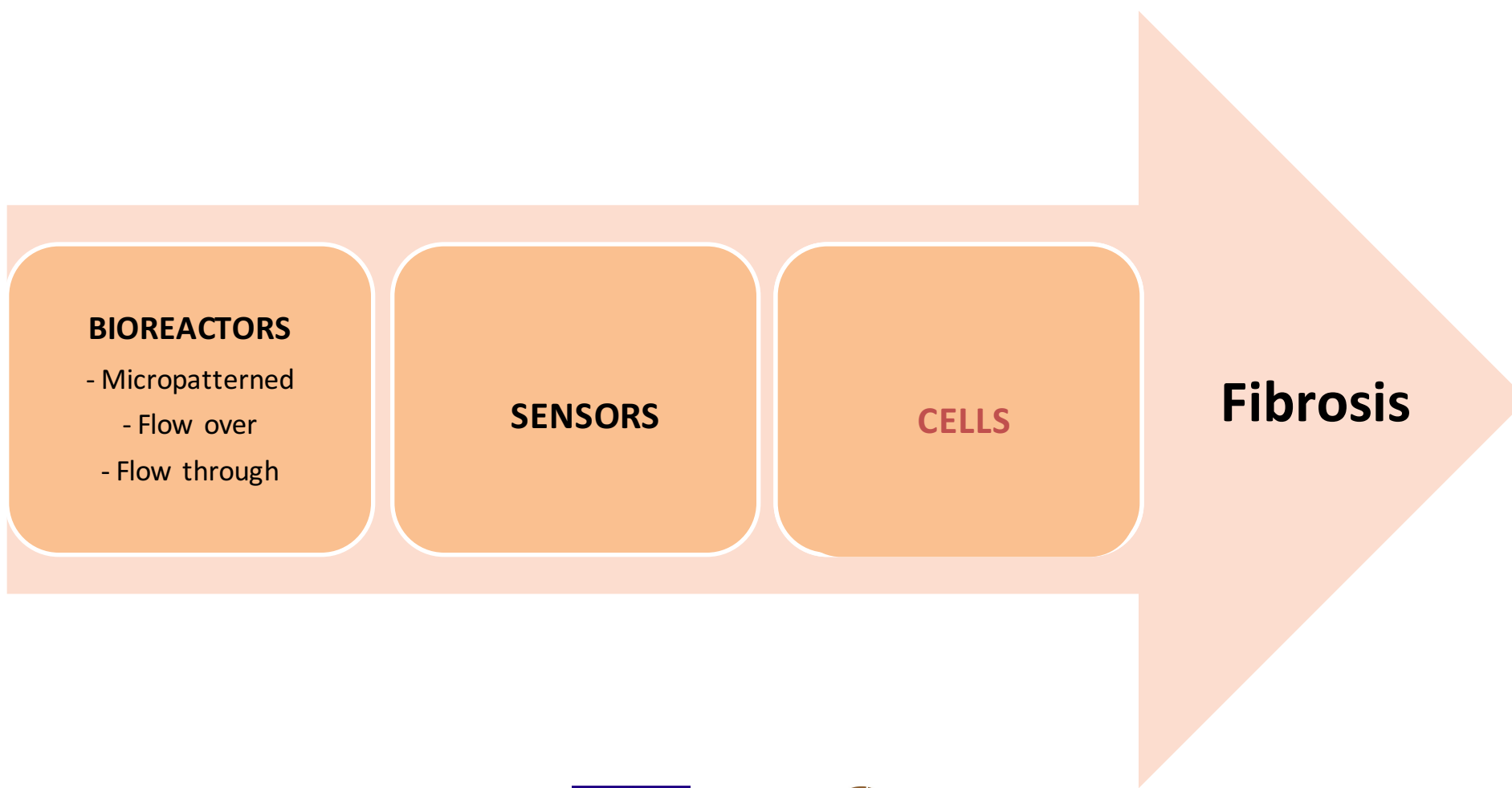
Single analyte solutions compared with a solution containing all analytes



Sequential exposure to the different analytes with no washing in-between

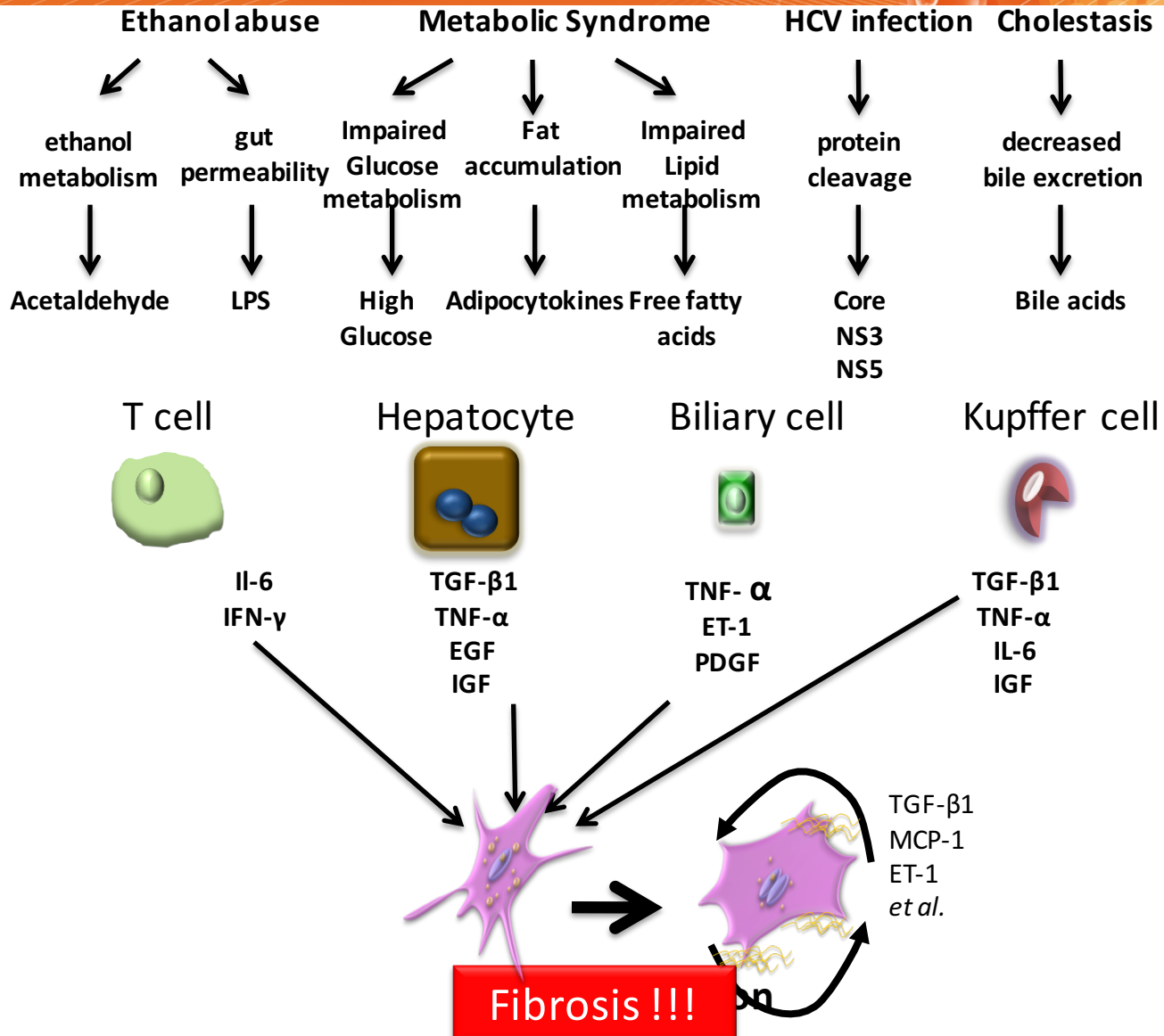




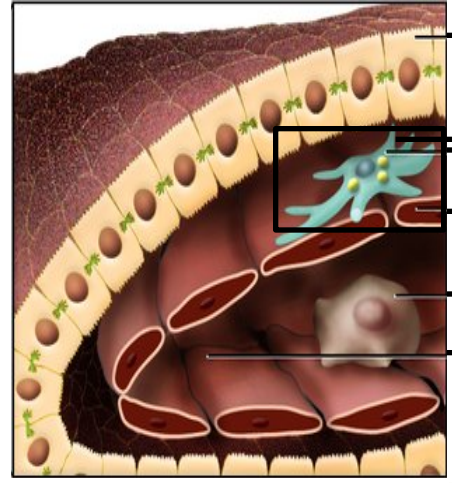


- Hepatocytes: with or without need for drug metabolisation
- Hepatic stellate cells?
- Hepatic sinusoidal endothelial cells?
- Immune and kupffer cells?

Depends on what one wishes to model!

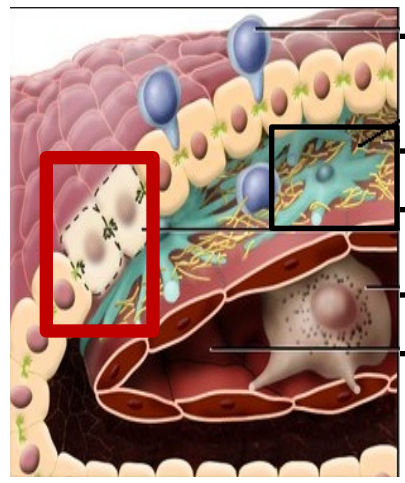


Healthy



- Hepatocyte
- Hepatic Stellate Cells (HSC) "quiescent"
- Sinusoidal endothelial cells
- Kupffer cell
- Sinusoid lumen with normal resistance to blood flow

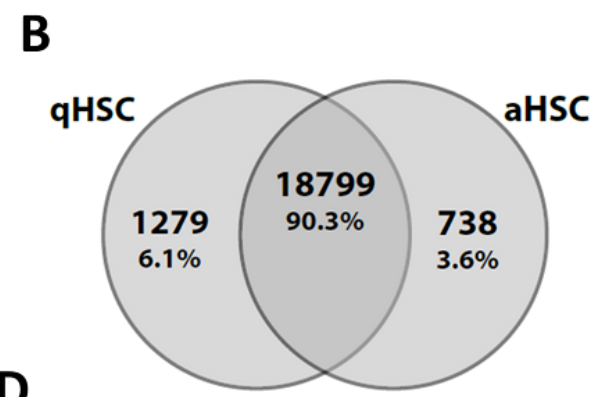
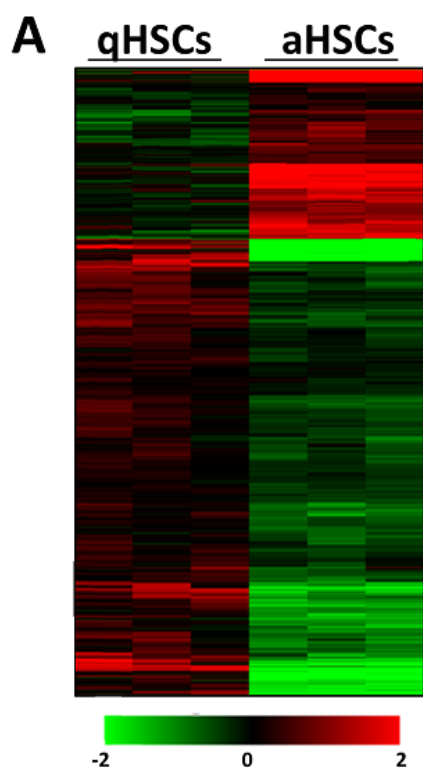
Fibrotic



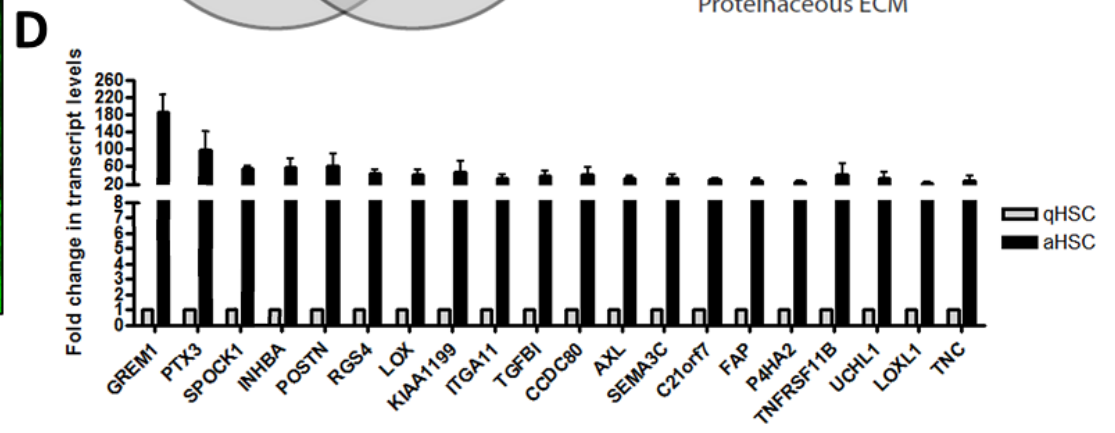
- Hepatic Stellate Cells (HSC) "activated"
- Infiltrating lymphocyte
- Extracellular matrix proteins
- Apoptotic hepatocyte
- Activated Kupffer cell
- Sinusoid lumen with increased resistance to blood flow



In vitro model?



- C** Most significant GO terms
- quiescent HSC**
 - Plasma membrane part
 - Immune system process
 - Cell periphery
 - activated HSC**
 - Extracellular matrix (ECM)
 - Biological adhesion
 - Proteinaceous ECM



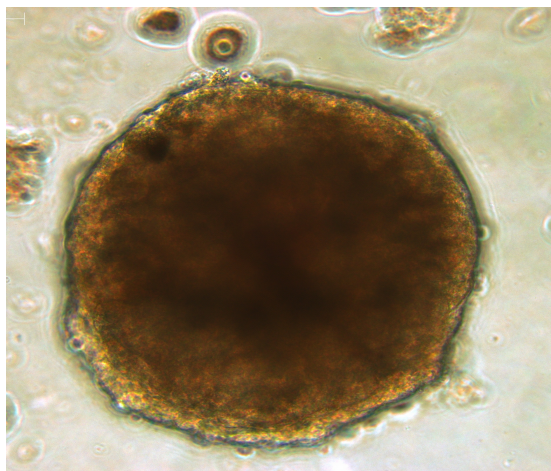
1. Characterisation

Cells to use

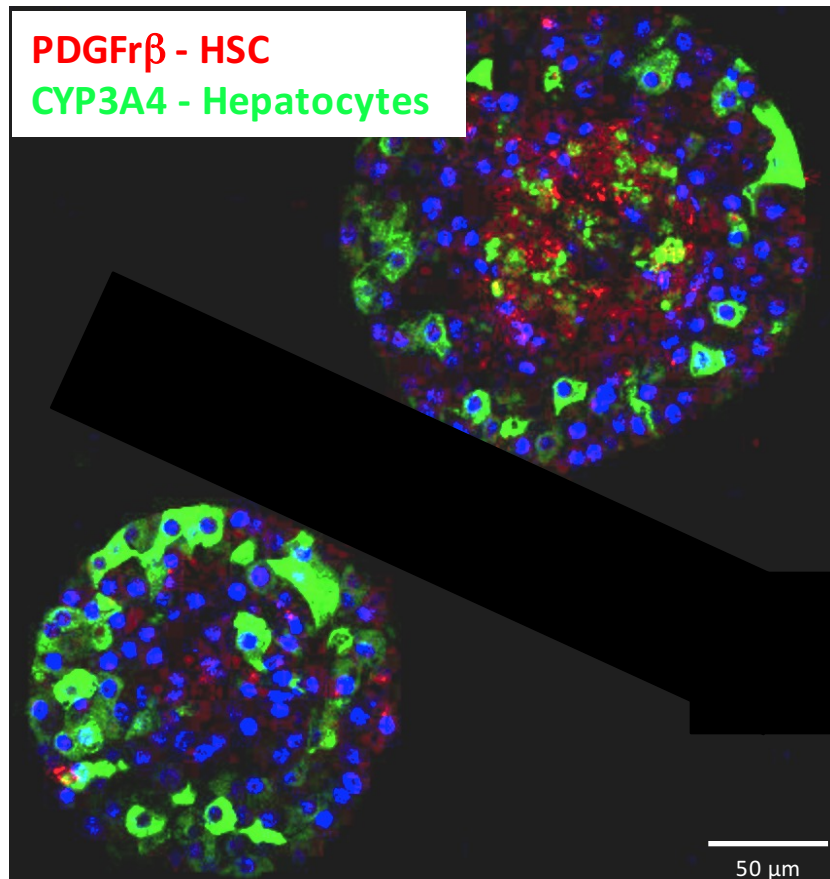
Cell ratio

Culture media

} 3D spheroids



Human primary stellate cells
Differentiated HepaRG (Biopredic)



1. Characterisation

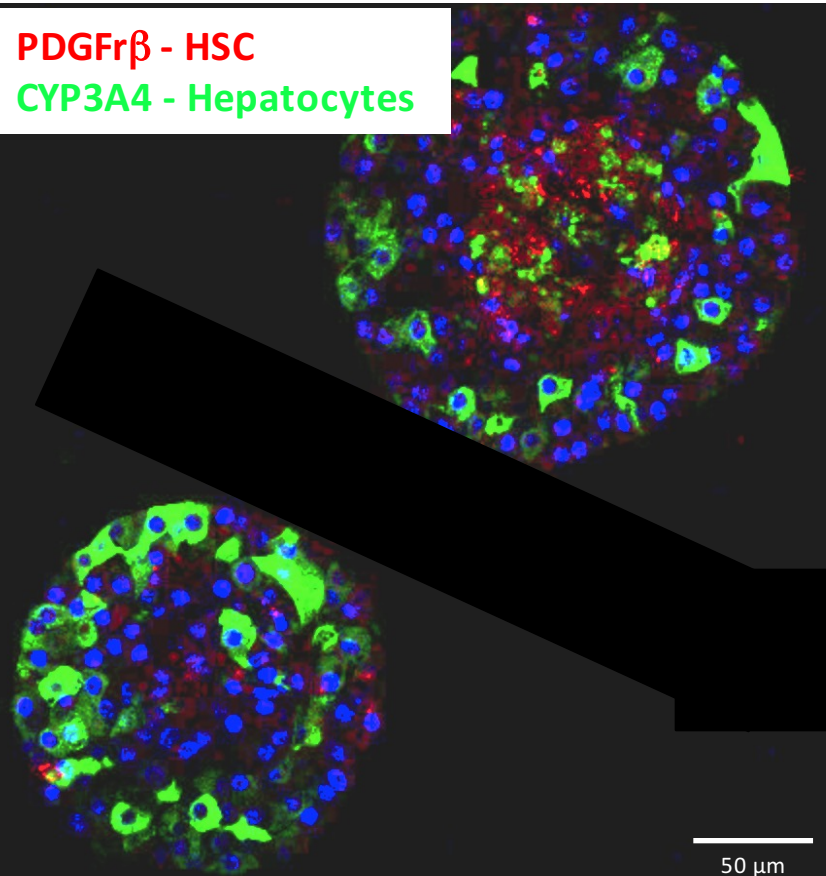
Cells to use

Cell ratio

Culture media

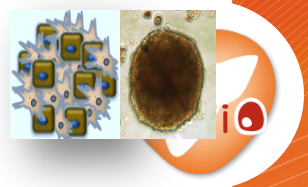
3D spheroids

Human primary stellate cells
Differentiated HepaRG (Biopredic)

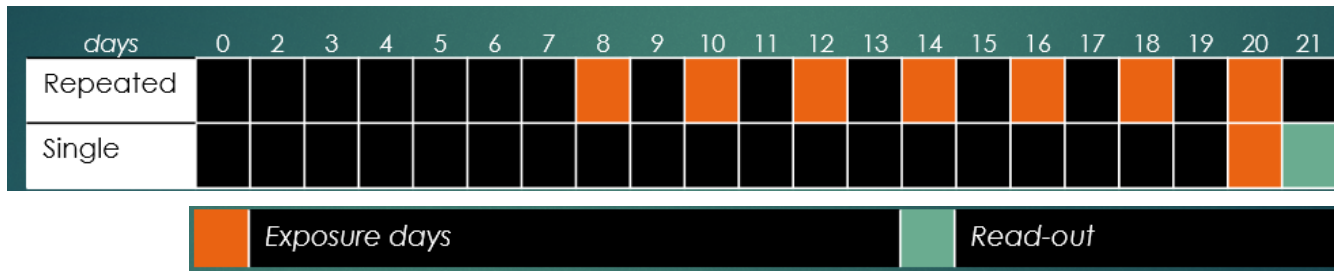


2. Testing fibrotic compounds

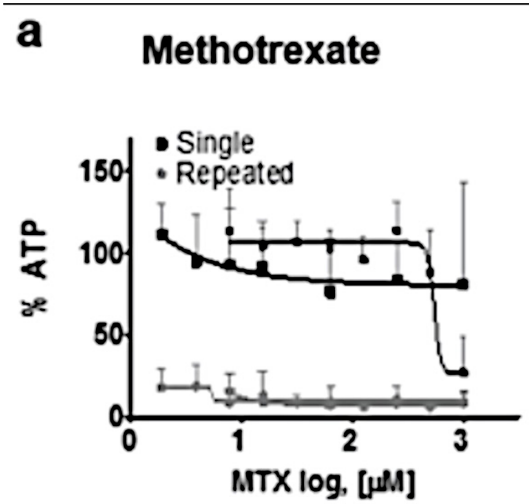
Methotrexate - MTX



Repeated Exposure vs Single Exposure : MTX

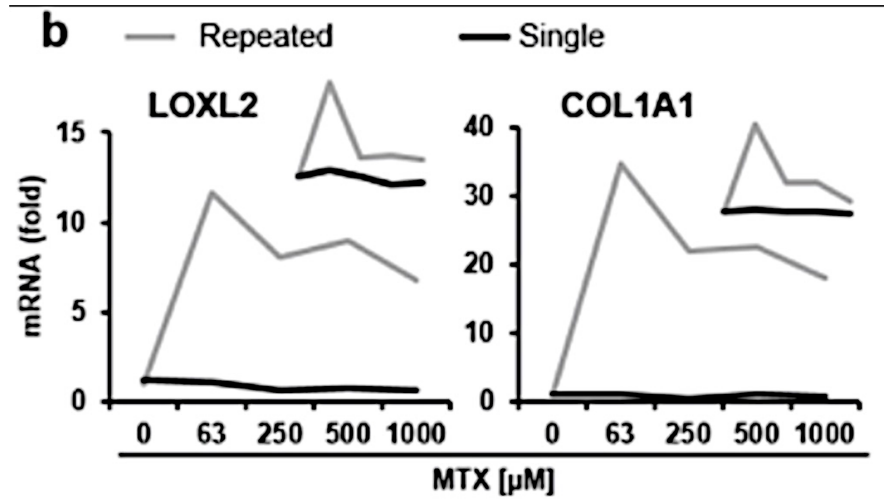


Cell Survival



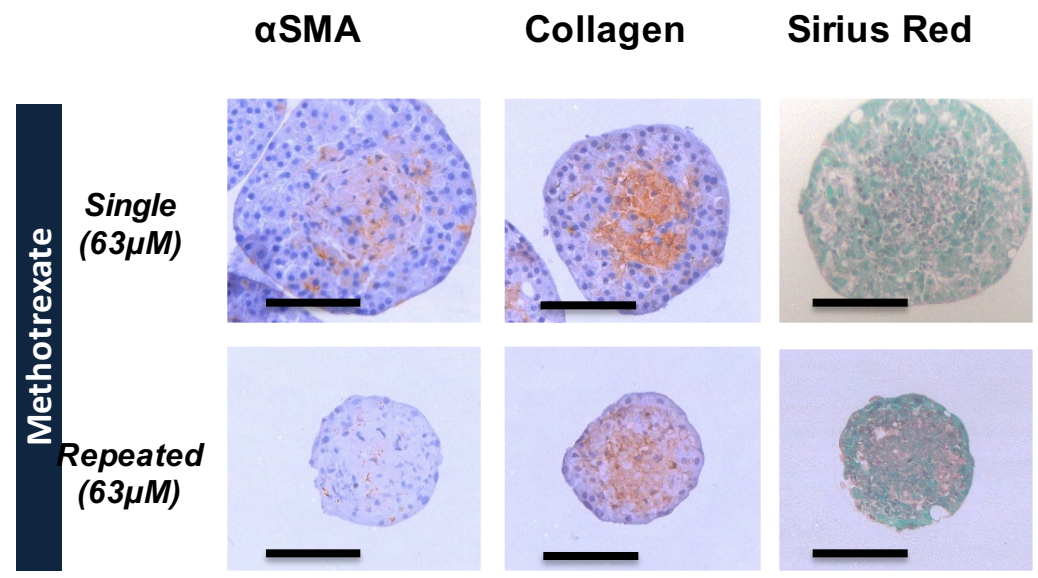
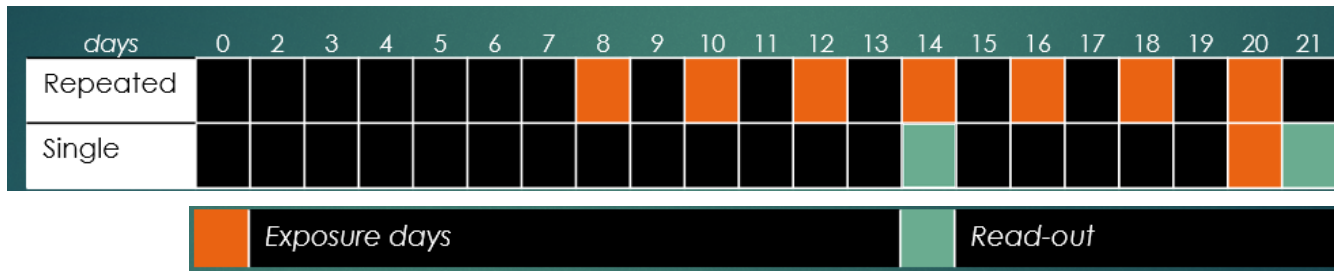
MTX is highly toxic after repeated exposures

Activation at the gene level

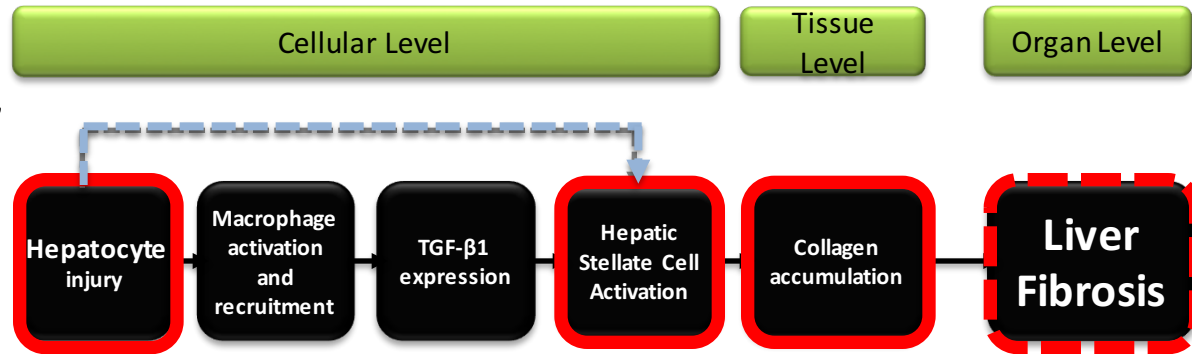


Repeated exposure to MTX induces a strong activation of HSCs

In vitro fibrosis?



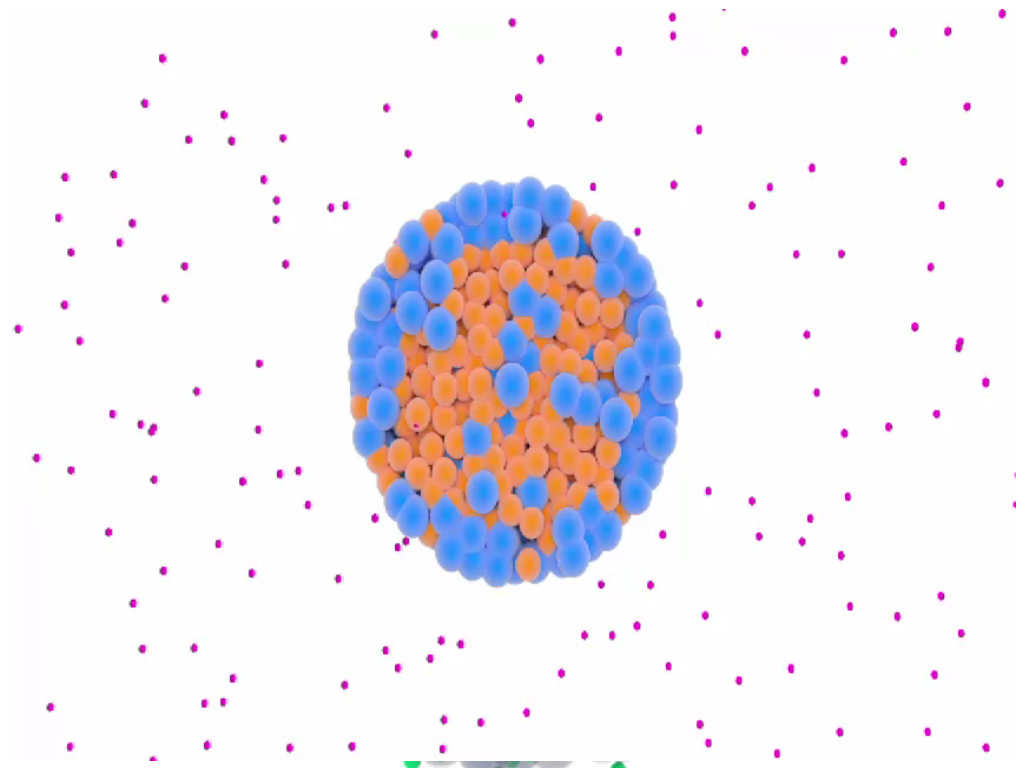
Repeated exposure to MTX induces fibrosis in vitro



Fibrosis *AOP*

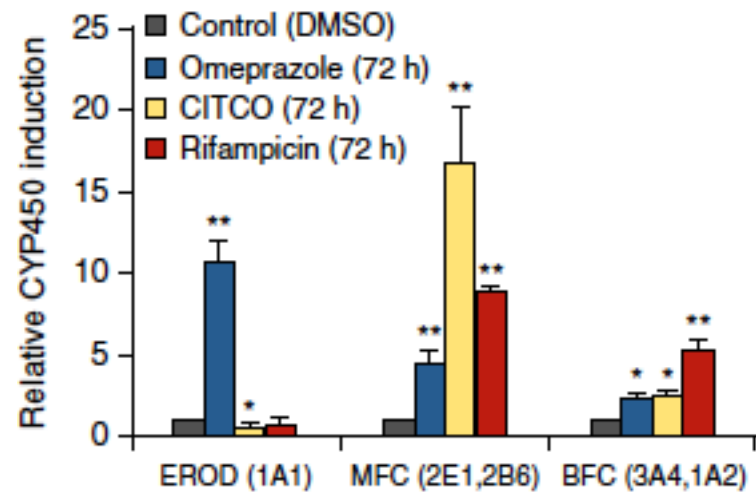
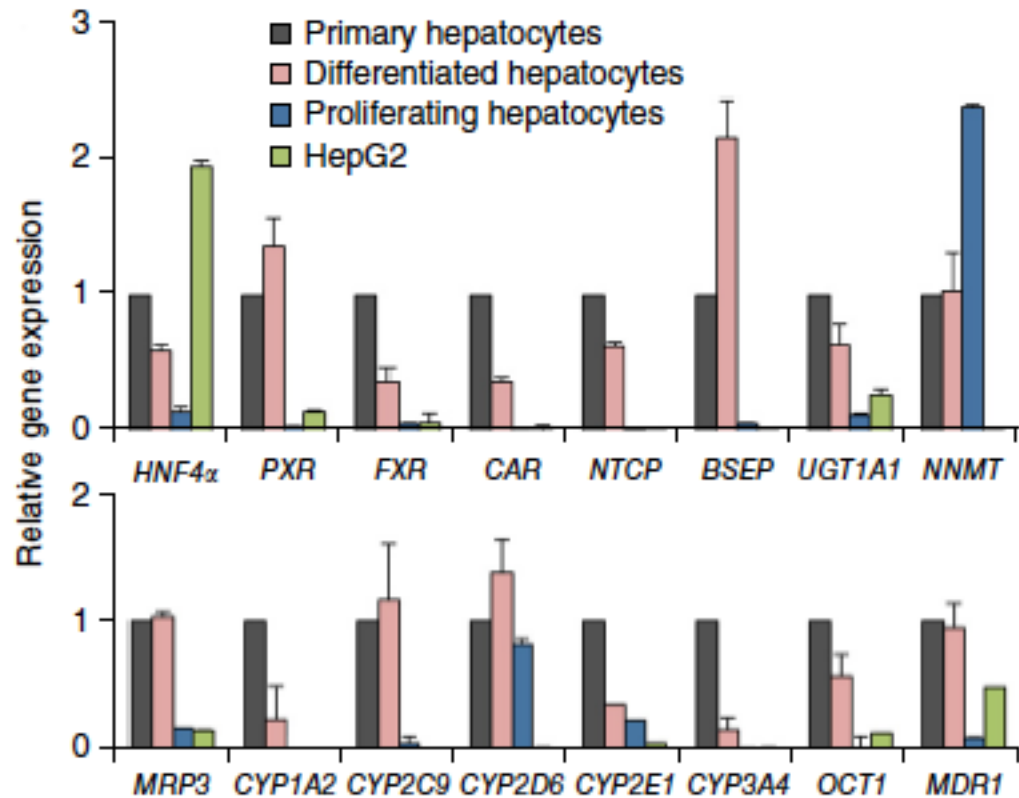
3D Hepatic co-culture

- Hepatocyte functional (>21 days)
- HSCs quiescent
- Responds to fibrotic compounds
- Single dose
- Repeated dose
- Compound dependent response



- UpCyte Hepatocyte
- iPSC-derived hepatocytes

Differentiated UpCyte hepatocytes have some of the mature hepatocyte functions

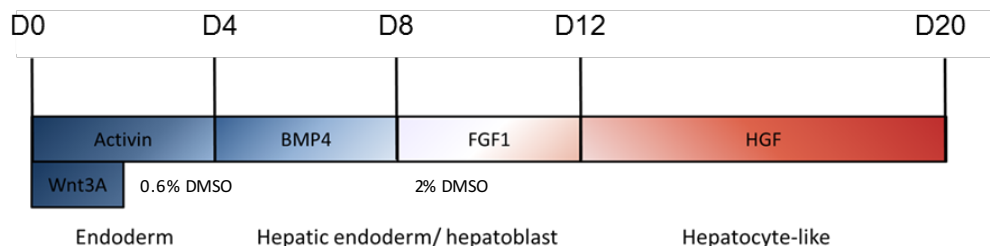


Alternative sources of hepatocytes

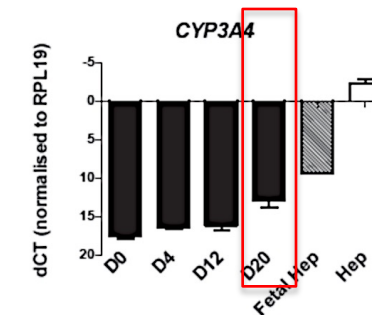
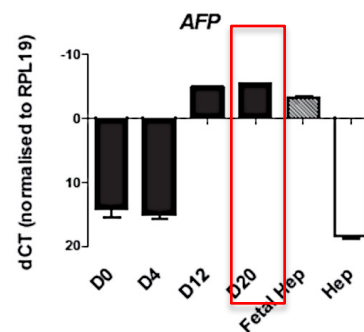
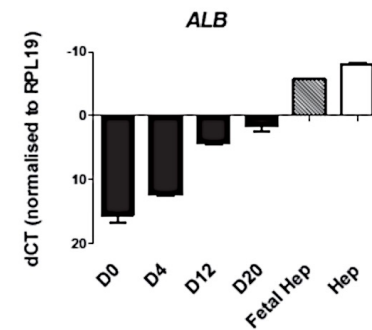
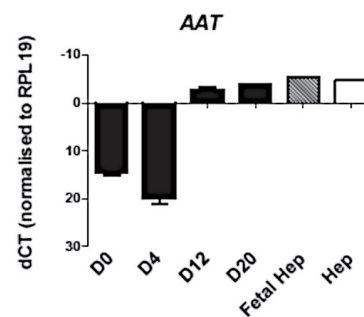
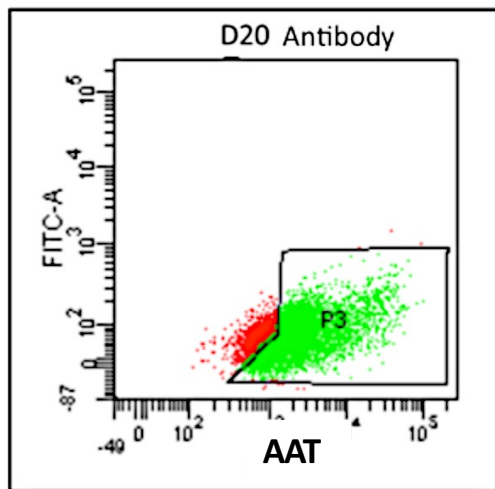
- UpCyte Hepatocyte
- iPSC-derived hepatocytes

- Is pluripotent
- Can thus, theoretically, generate all cells needed to model fibrosis
- Can be generated from multiple donors, reflecting differences in metabolism and toxicity

**But, can we generate hepatocytes
(other cells) from PSC?**



<i>OCT4</i>	<i>CXCR4</i>	<i>ALB</i>	<i>ALB</i>
<i>SOX2</i>	<i>CKIT</i>	<i>AFP</i>	<i>AAT</i>
<i>NANOG</i>	<i>FOXA2</i>	<i>HNF4α</i>	<i>CYP3A4</i>



Hepatocytes from hiPSCs: can we learn something from transcriptomic studies compared with development?

Bioreactor

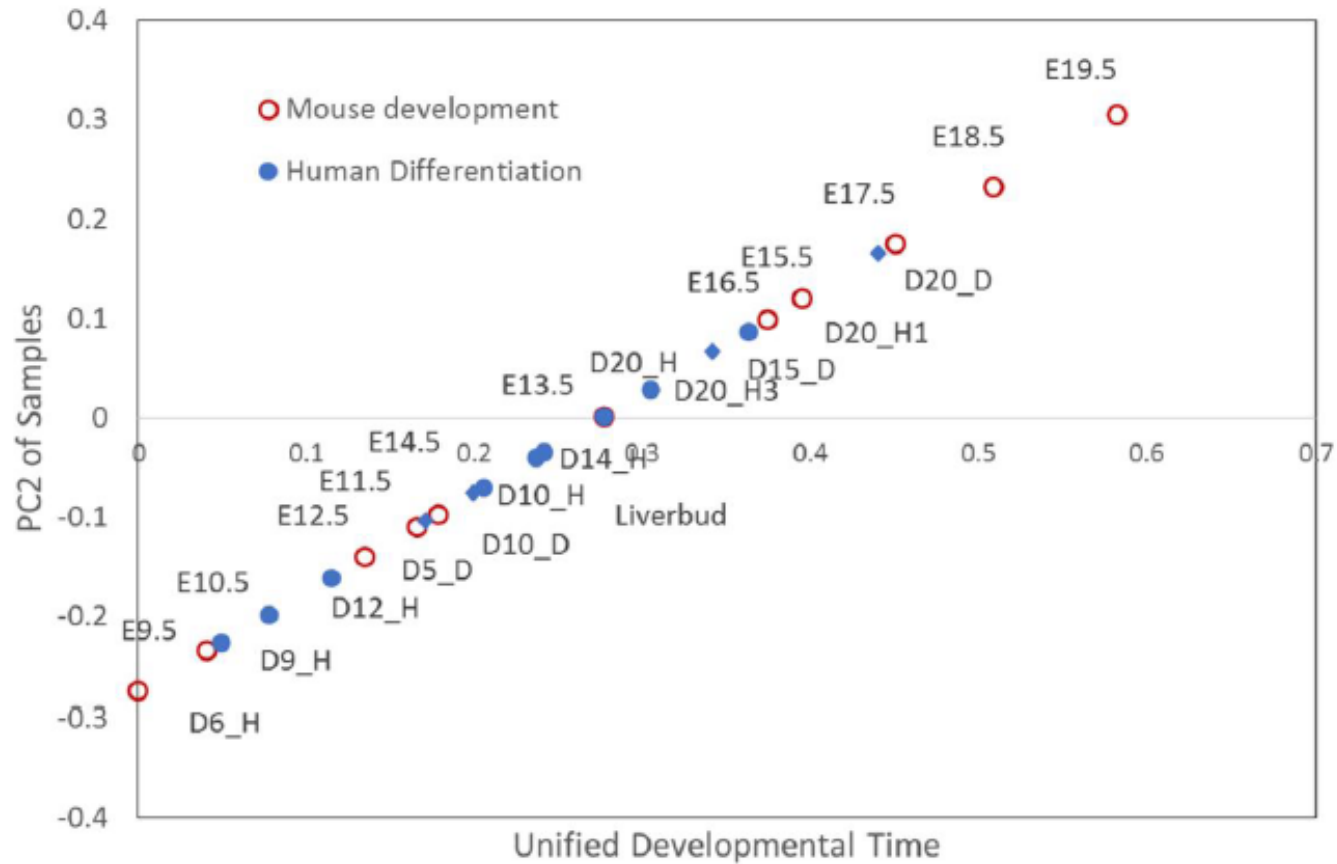
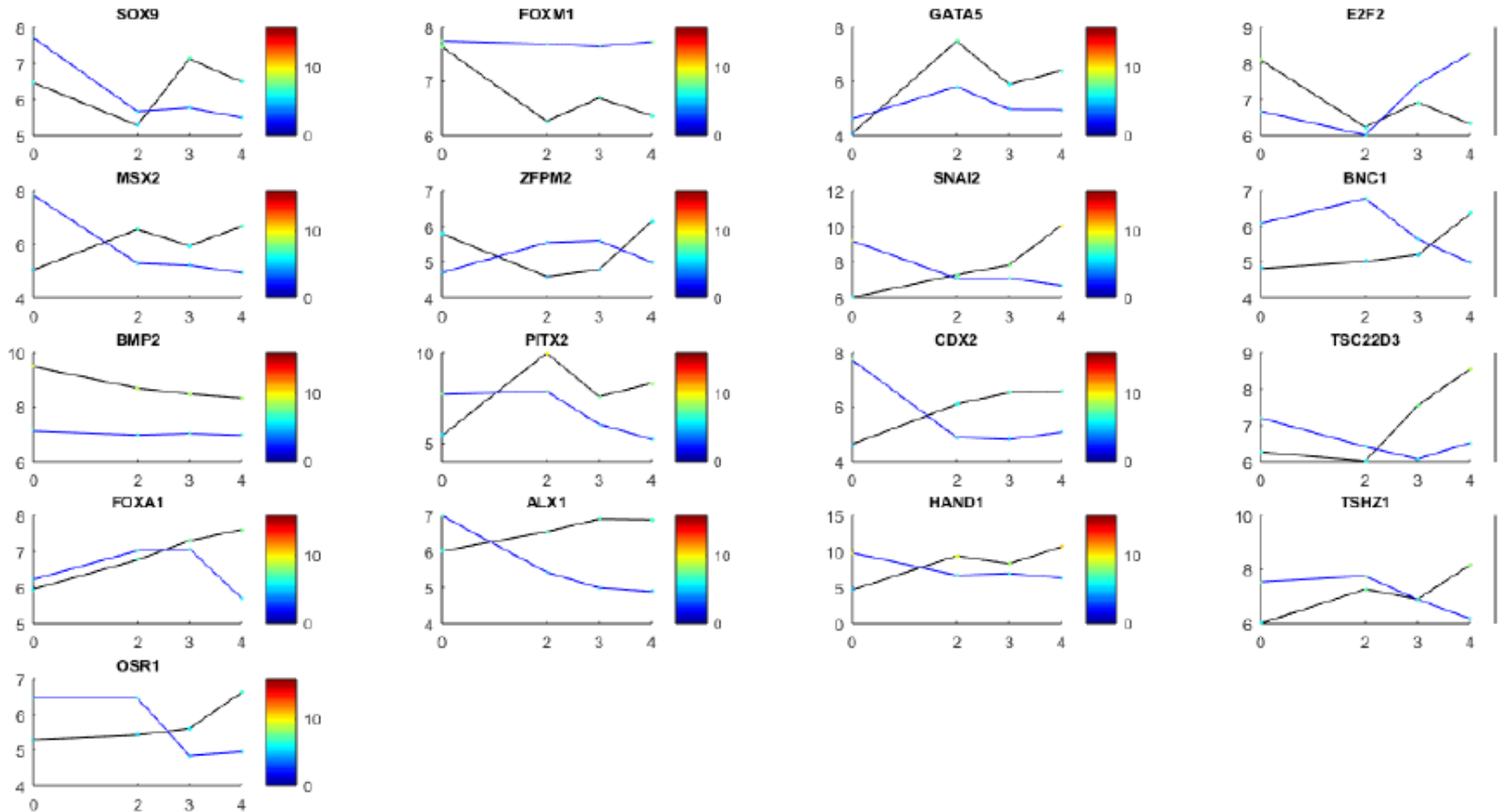


Figure 7-18: The PC2 values for mouse and human samples are plotted against their unified developmental time (DT). The DT increases with increase in maturity, and most HLCs fall within DT corresponding to E15.5

Hepatocytes from hiPSCs: many transcription factors are not expressed or incorrectly expressed

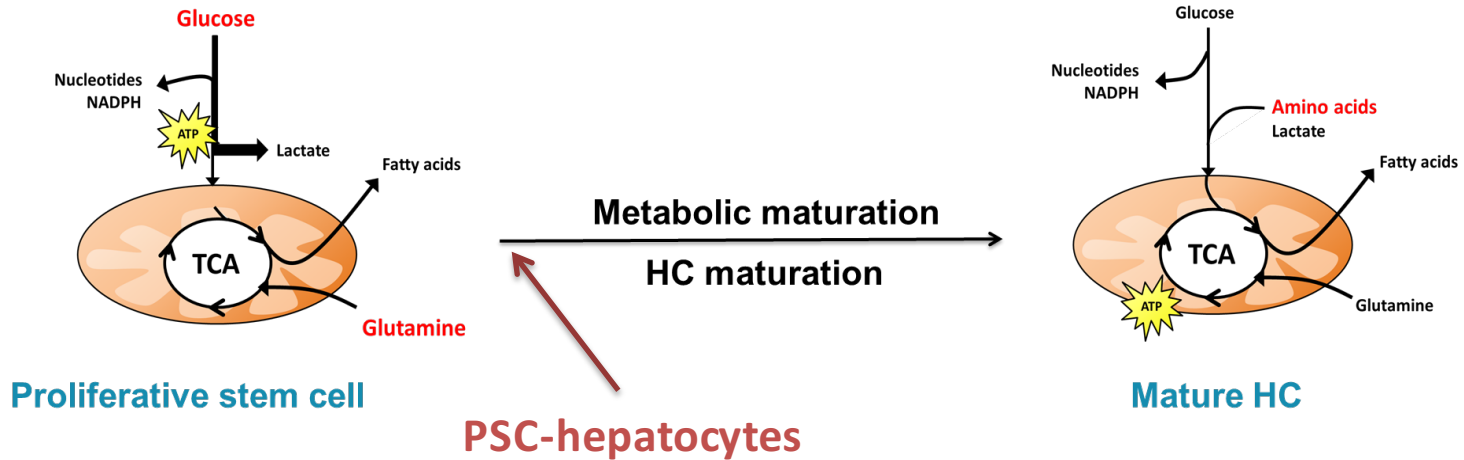
Bioreactor



Blue: Mouse development (E9.5,E11.5,E13.5,E15.5 and E19.5)

Black : Hu Differentiation (D6,D10,D14,D20,D20)

In contrast to primary hepatocytes, iPSC hepatocytes still use glycolysis



- High glycolysis
- Low mitochondrial content
- Glucose and glutamine dependent

- High OXPHOS
- High mitochondrial biogenesis
- Unlabeled carbon sources

PGC-1 α is the master regulator of mitochondrial biogenesis
Is only minimally expressed in PSC-hepatocytes

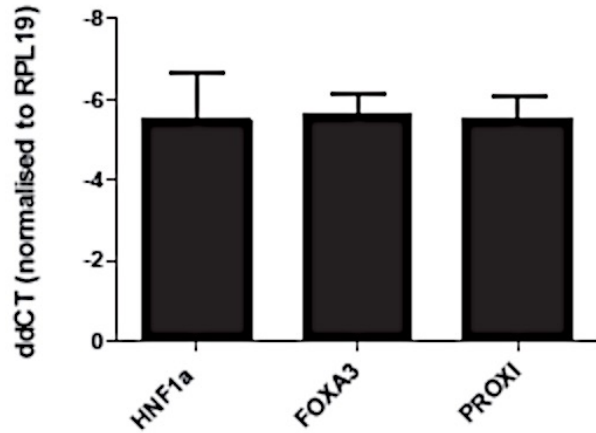
Overexpression of missing transcription

Overexpression of HNF1a_FOXA3_Prox1 induces ALB and CYP3A4, but does not decrease AFP → still immature

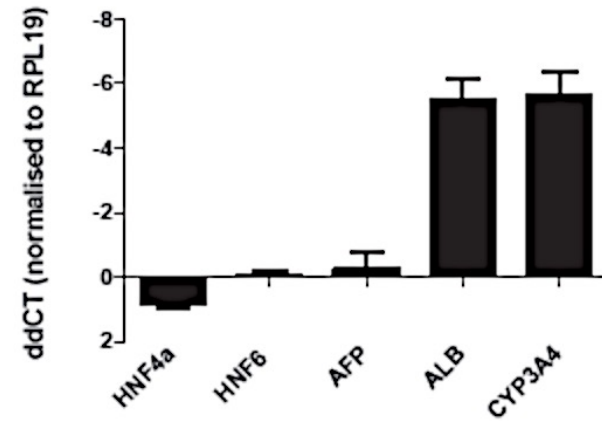
Bioreactor



Rel expression dox vs CTL

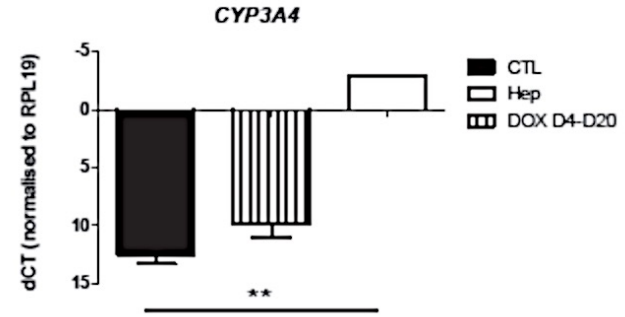
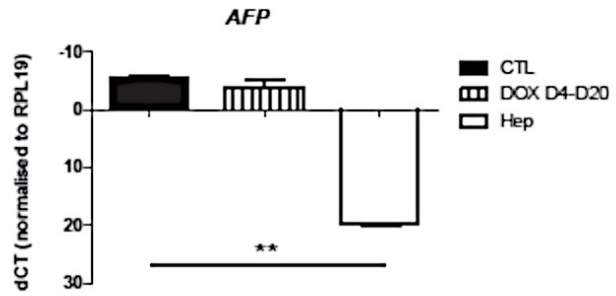
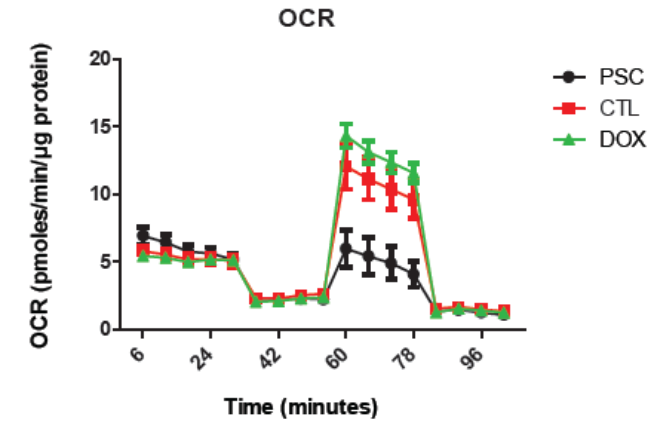
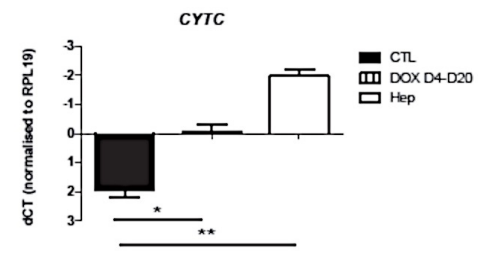
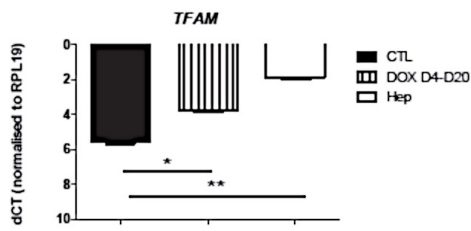
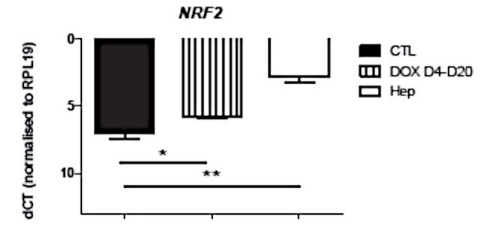
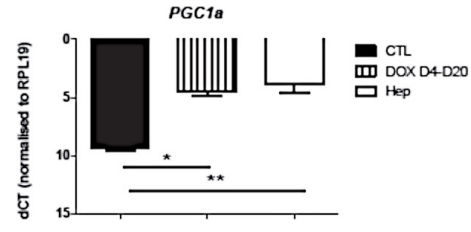


Rel expression dox vs CTL



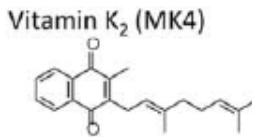
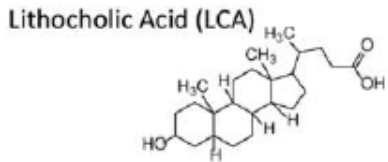
Overexpression the master-regulator of mitochondrial biogenesis, PGC-1 α , increases OxPhos (some) \rightarrow still immature

BioFactor



- Induction of missing TFs seems not to be sufficient to create mature hepatocytes
 - Can combined activation of missing TFs and repression of incorrectly expressed TFs accomplish this?
- Differentiation methods not good enough!
 - Growth factors, medium changes?

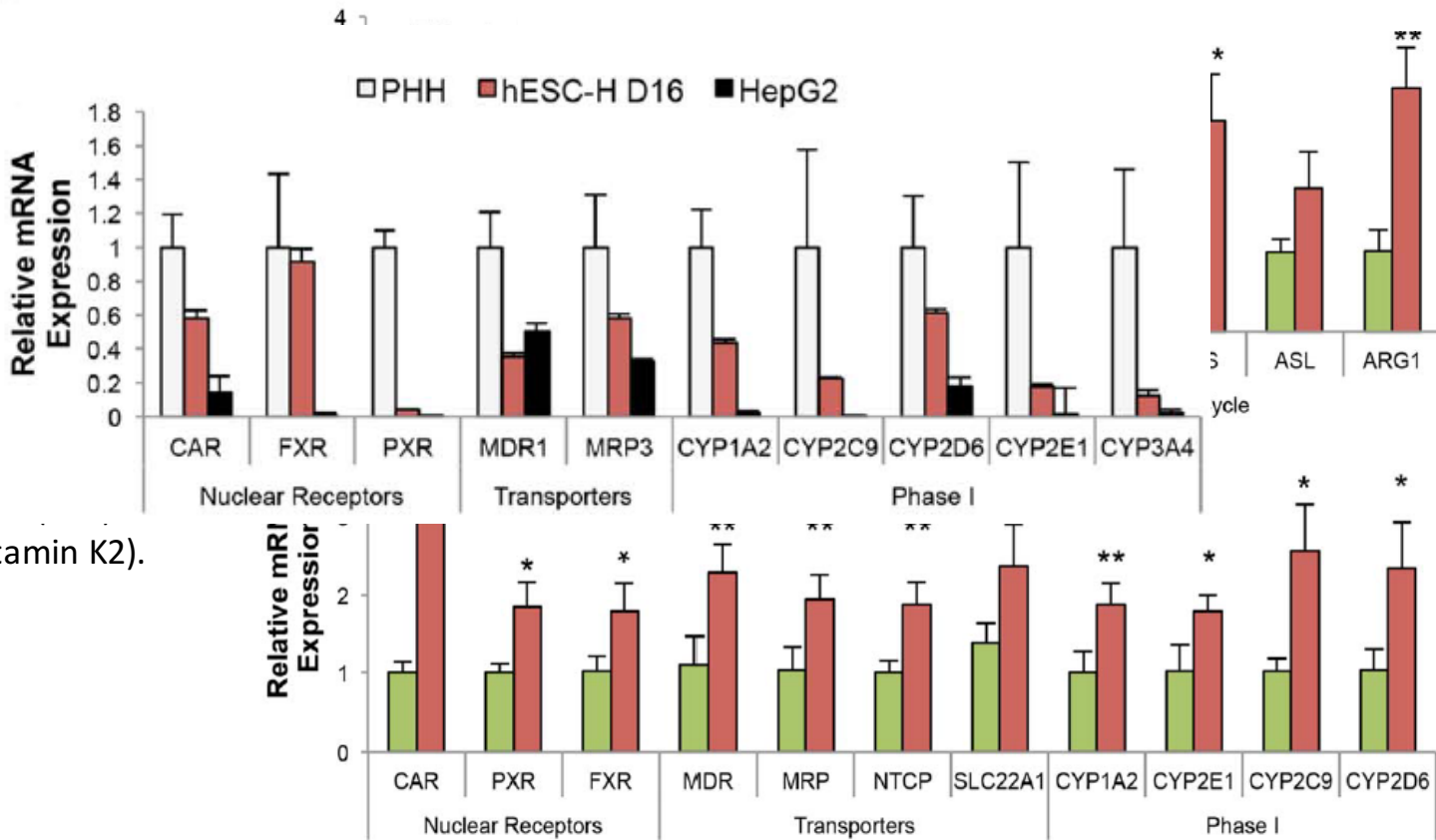
Addition of LCA and vitamine K (present postnatally) induces (some) differentiation → still immature



Postnatal

fatty acids from primary energy

gut colonization leads to others lithocholic a menaquinones (vitamin K2).



- Induction of missing TFs seems not to be sufficient to create mature hepatocytes
 - Can combined activation of missing TFs and repression of incorrectly expressed TFs accomplish this?
- Differentiation methods not good enough!
 - Growth factors? Incremental improvement
- Attempt to make only hepatocytes (or HSCs, LSECs) incorrect: i.e. create in combination!
- Differentiations commonly done in plastic dishes, in 2D; which is known to cause very fast de-differentiation of primary hepatocytes

✧ **3D in bioreactor!!**

To address fibrosis, HeMiBio has

Bioreactor

- created several microfluidic bioreactors that can be used for long-term liver toxicity testing as well as to optimize long-term hepatocyte culture
- created extracellular sensors that can assess liver cell toxicity
- created master PSC lines that allows very fast introduction of Tox sensing cassettes
- developed an in vitro model that can assess fibrosis in a medium-term toxicity setting using HepaRG and primary stellate cells
- tested multiple candidate hepatocyte populations (UpCyte and PSC derived) as genetically more diverse alternatives for HepaRG cells; however all still fall short of primary hepatocytes



HeMiBio



UNIVERSITY OF OSLO



Vrije Universiteit Brussel



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London, October 26-27, 2015

