CRACK IT

CRACK-IT Challenge: Improved *in vitro* to *in vivo* extrapolation in chemical safety risk assessment of human systemic toxicity

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NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

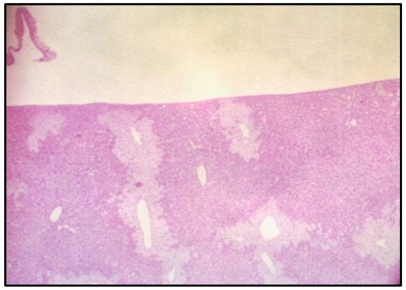
LIVERPOOL JOHN MOORES UNIVERSITY

Workshop: Applying exposure science to increase the utility of non-animal data in efficacy and safety testing

Drug-Induced Liver Injury

- ***Leading cause of acute liver failure*[1]**
- *High morbidity & mortality*[2]
- *Main reason for late stage termination or withdrawal*[2]
- *76 drugs found to be significant cause of hepatotoxicity across 3 DILI Registries (US, Sweden, Spain)*[3]

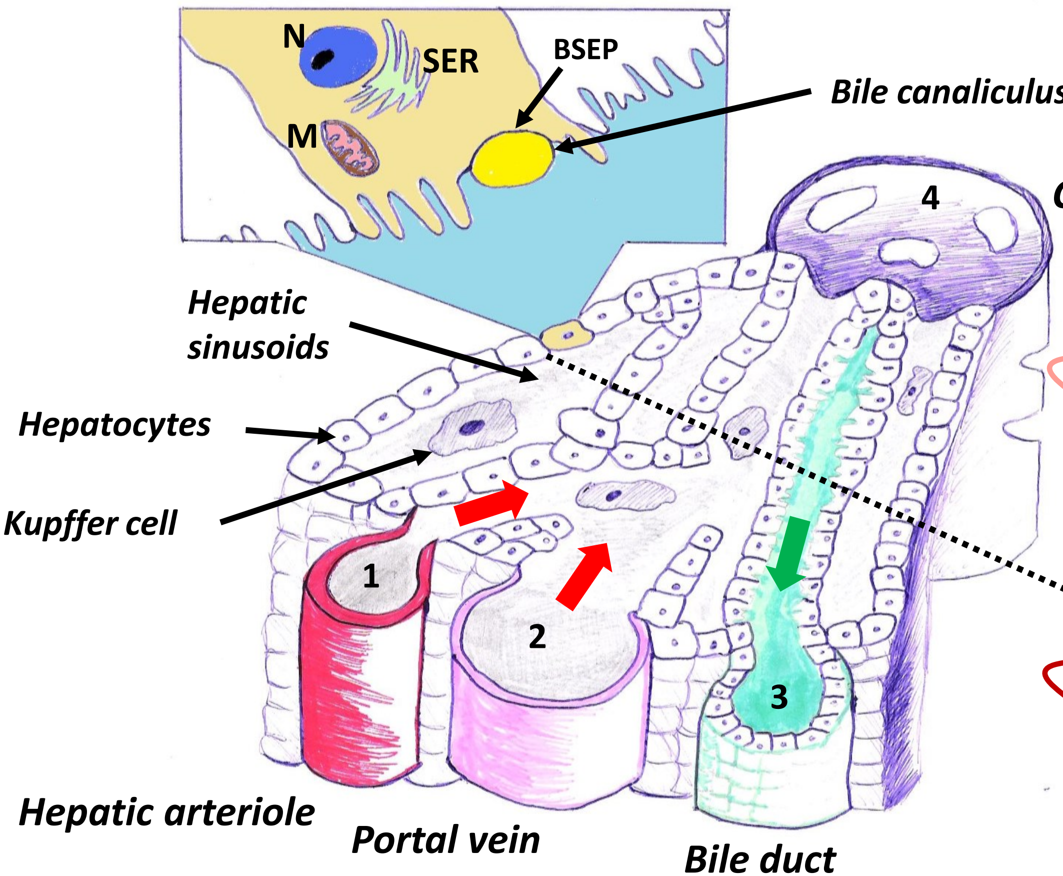
- ***Current in vitro technologies:***
  - *Physiological gap between incubations and liver*
  - *Lack of physiological integration for amplification/adaptation*
  - *Inability to assess how minor chemical stress leads to major toxicity in some people*



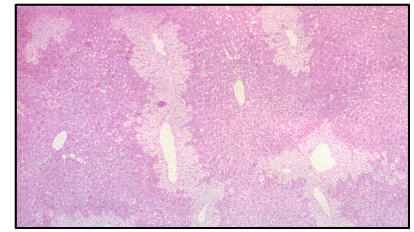
Require novel translational *in vitro* models of hepatoxicity

[1] Lee AASLD, 2009; [2] Verma & Kaplowitz 2009; [3] Suzuki et al., 2010

Replicating Liver Physiology for toxicology



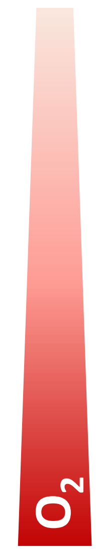
Paracetamol (mouse)



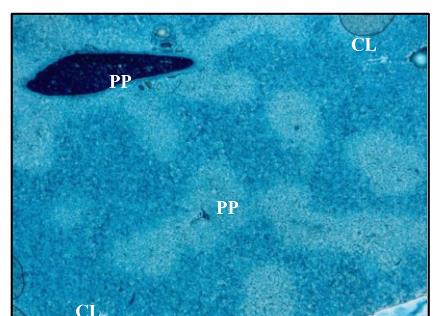
Perivenous / Centrilobular
↓ Oxygen
↓ Hormones
Glycolysis
Chemical detoxification
Lipogenesis

Periportal
↑ Oxygen
↑ Hormones
Gluconeogenesis
Ureagenesis

~4%

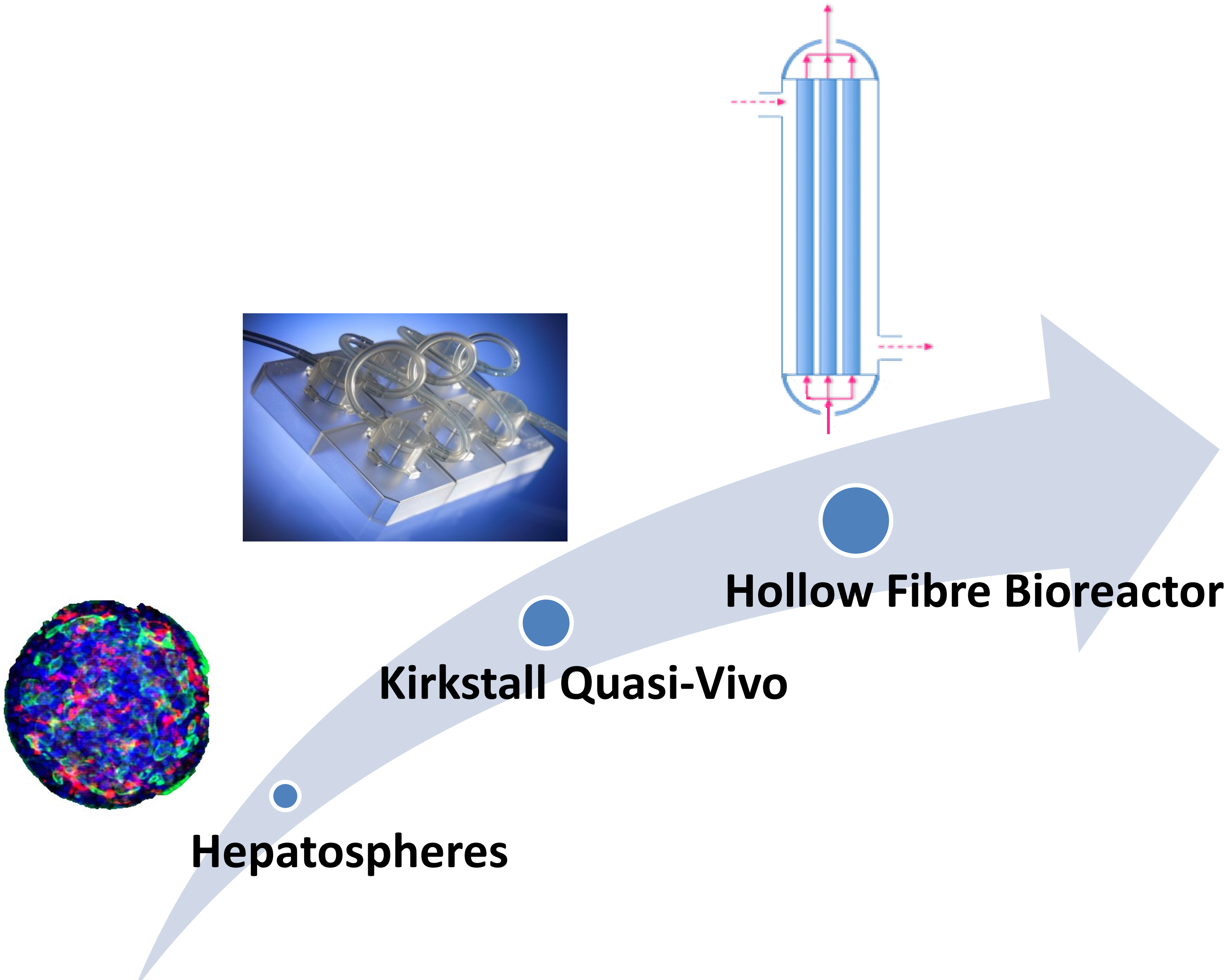


~13%

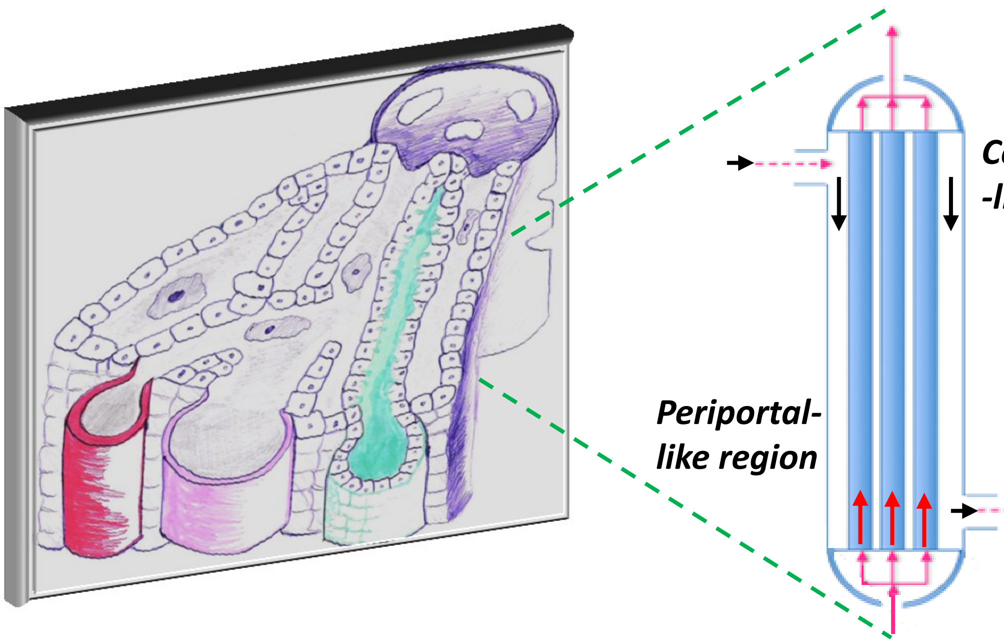


Methapyrilene (rat)

Mathematical modelling to improve and optimize the design of 3D liver in vitro models



An in vitro hepatic sinusoid: hollow fibre bioreactor



- 3D cellular scaffolds
- Multiple cell-types from same organ
- Bioreactor environment allows physiological recapitulation
  - Hepatocyte zonation
  - Bile contra-flow
- Adaptation to & amplification of response
- Improved ADME vs current models

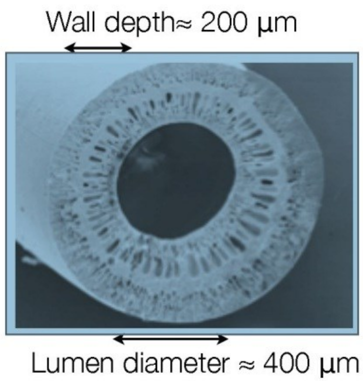
Cell number
Viability
Morphology

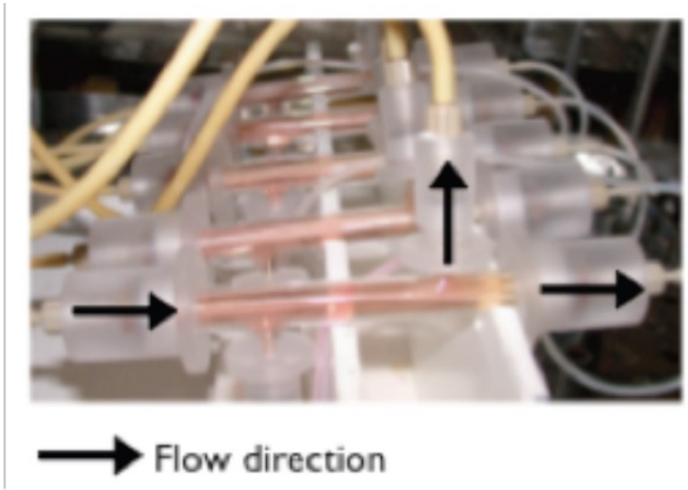
Oxygen level
Pressures
Flow rates

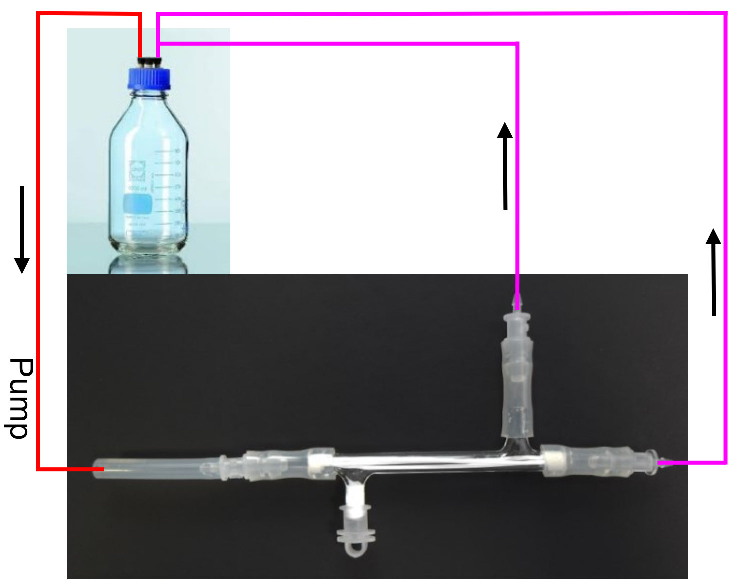
Glucose
Albumin
Urea
Glycogen

Challenge 5: IVIVE https://www.crackit.org.uk/challenge-5-ivive

Hollow Fibre Bioreactor







Disadvantages:
- *Fiddly to set-up*
- *Low throughput*

Advantages:
- *In-vivo like culture system*
  - *Its possible to generate oxygen and nutrient gradients*
- *Cells are shielded from shear stress*
- *Superior mass transport*
- *High cell densities*

Develop a zonated hepatic hollow fibre bioreactor for chemical safety assessment

National Centre for the Replacement Refinement & Reduction of Animals in Research

Cathy Vickers

syngenta

Richard Currie
Pratibha Mistry

Unilever

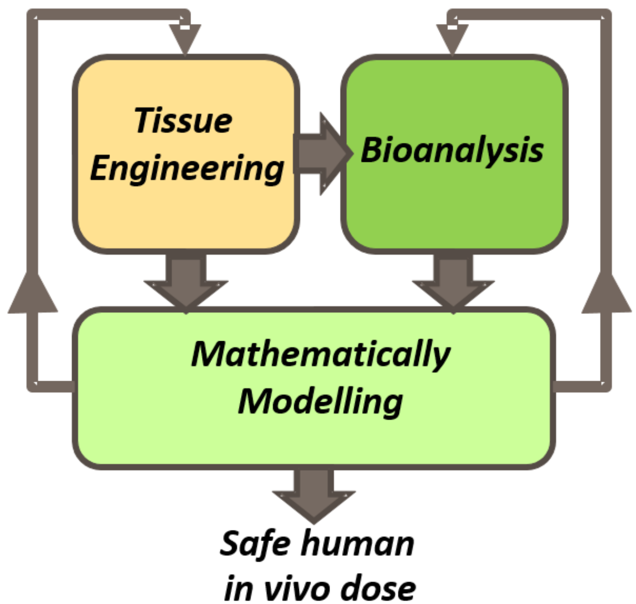
Andrew Scott
Ian Sorrell

AstraZeneca

Dominic Williams
Sophie Regan
Domingo Salazar

University of Bath

Marianne Ellis
Mike Storm
Kim Leutchford



University of Liverpool

Parveen Sharma
James Firman

Liverpool John Moores University

Steve Webb

UCL

Rebecca Shipley

SIMCYP
real solutions from virtual populations

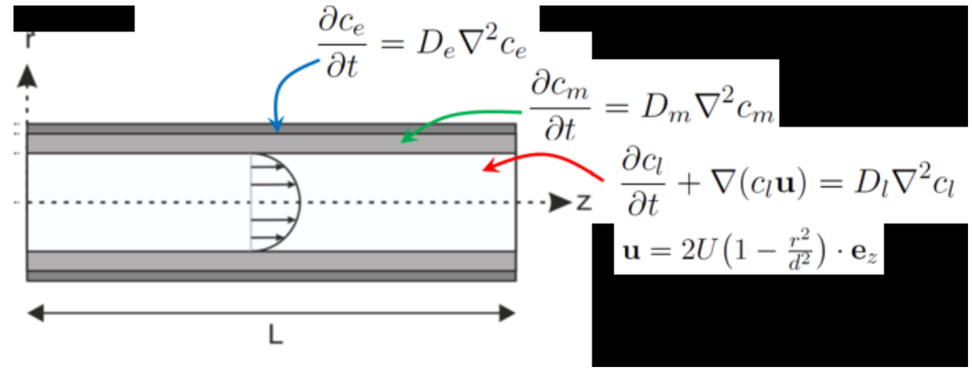
Iain Gardner

Loughborough University

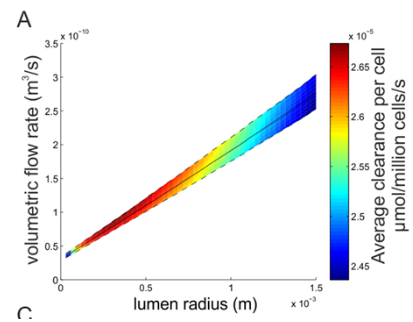
John Ward

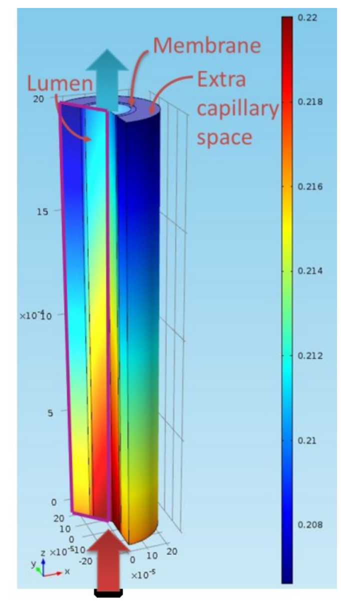
Role of Mathematics

- ***Aid design and development***
- *Set operating conditions*
- *Experimental design*

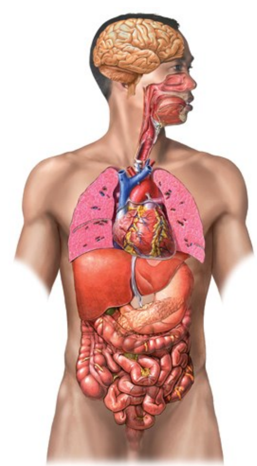


- ***Interpret data***
  - *Compare other in vitro systems*
  - *Aid quantification*

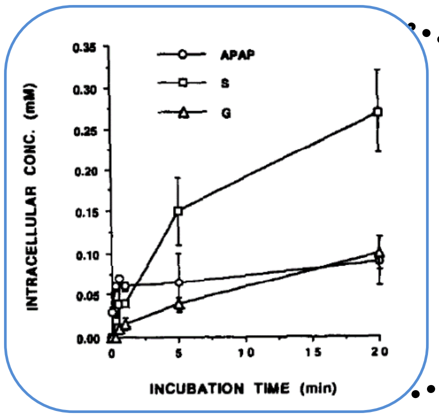


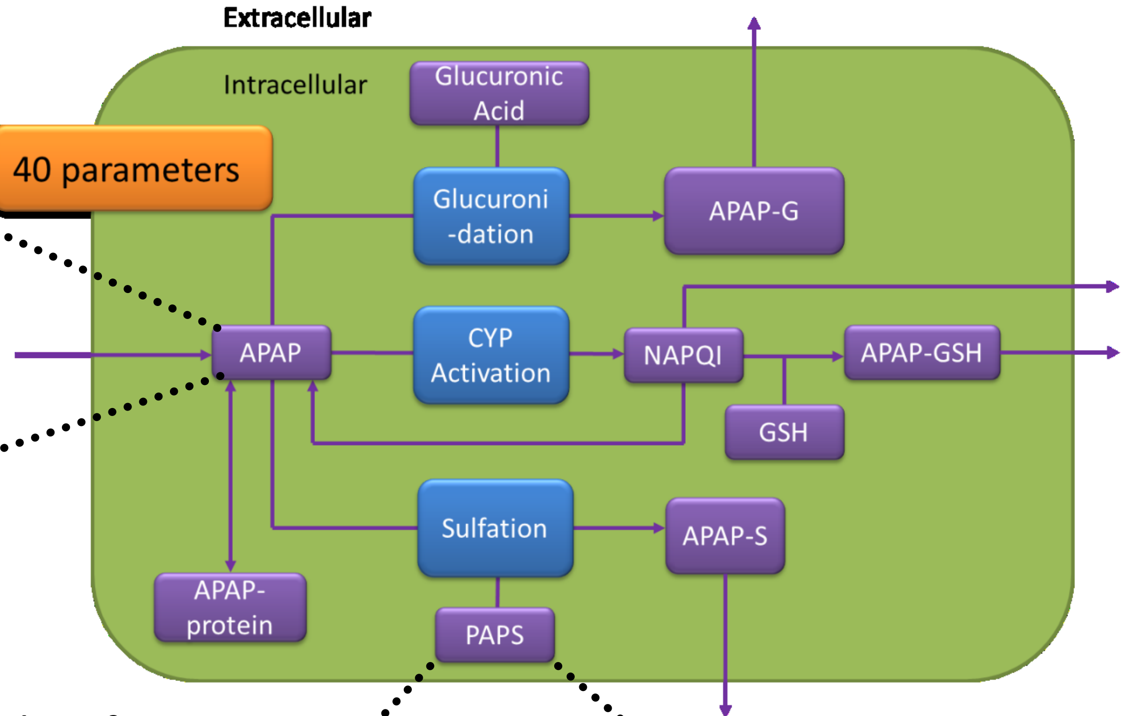


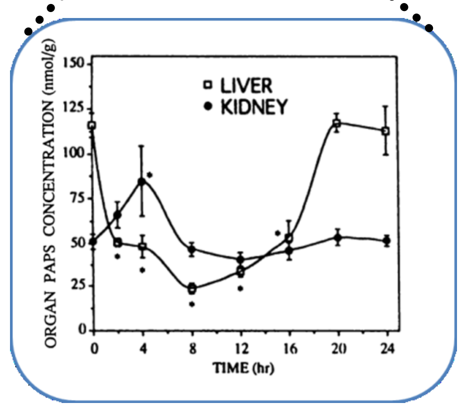
- ***Extrapolate from in vitro to in vivo***



In silico hepatocyte



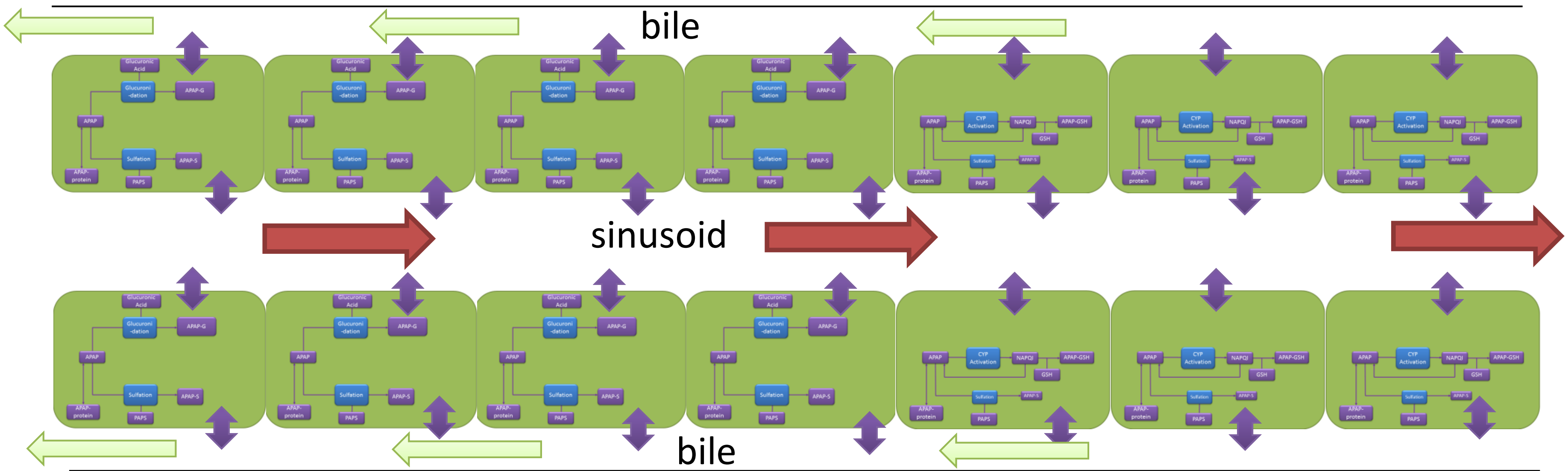




- Parameterized & validated using data from in house and literature
- Modelling directs ‘wet-lab’ research
- Allows visualization of enzyme capacity

Kim *et al.*, 1992; McPhail *et al.*, 1993

In silico hepatocyte sinusoid



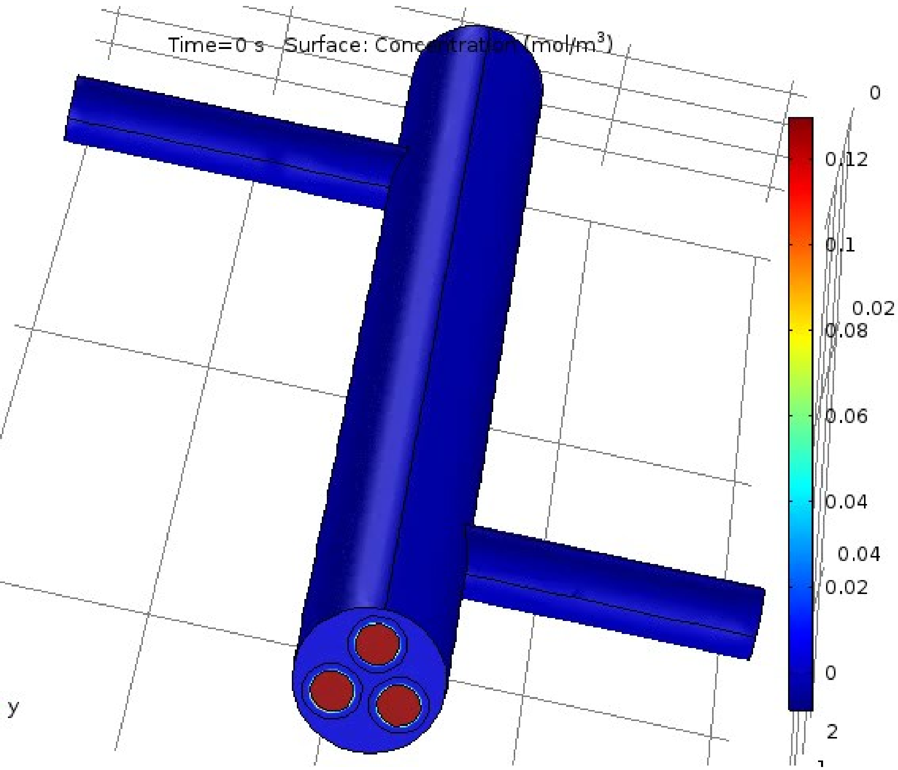
HFB Considerations:

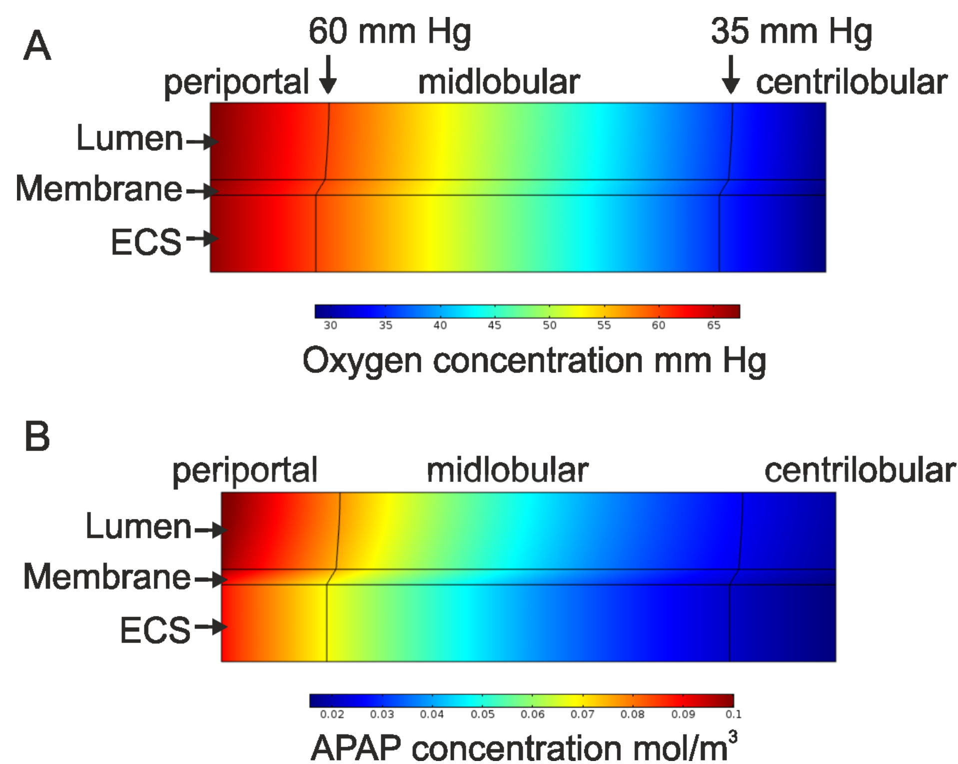
Physical dimensions
Membrane properties
Flow rate
Inlet oxygen concentration

Cell considerations:

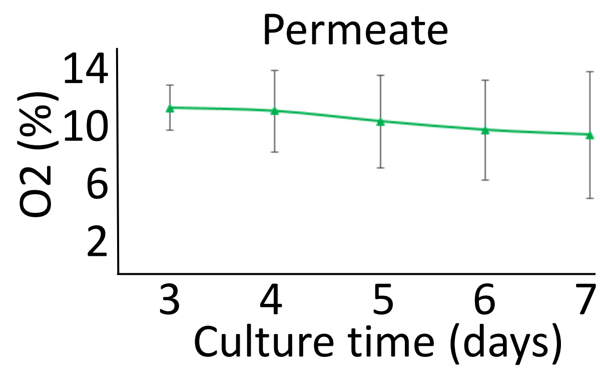
Density
Oxygen consumption
Drug uptake/metabolism

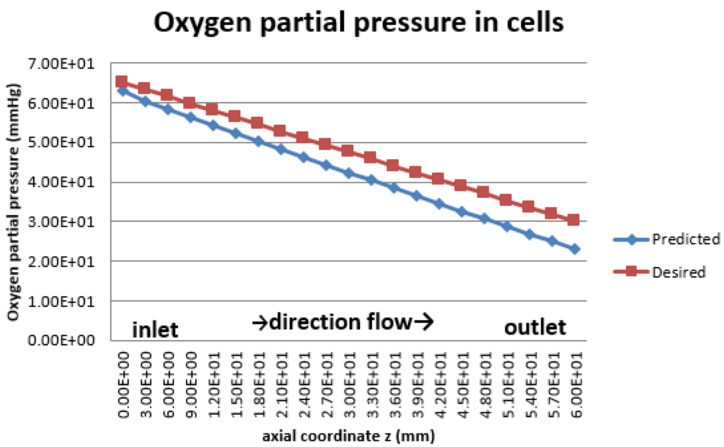
Oxygen and drug transport in HFB

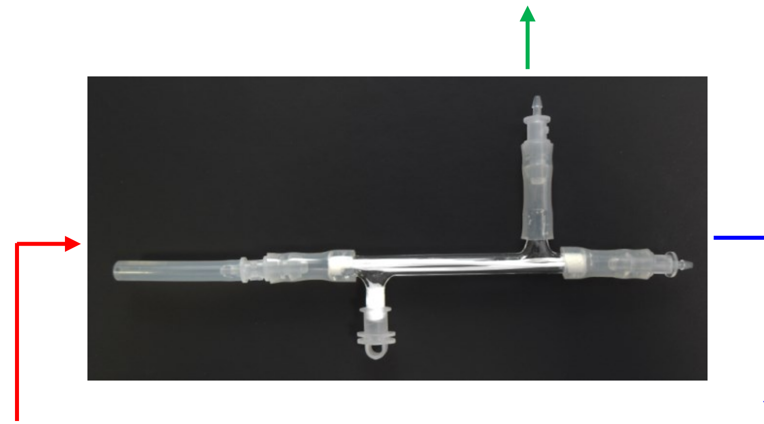




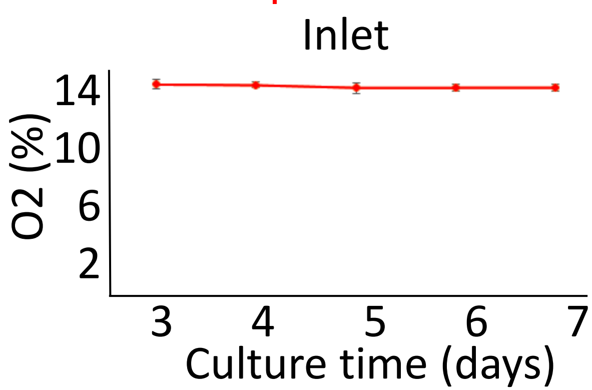
Mathematical predictions – Oxygen Gradient in HFB

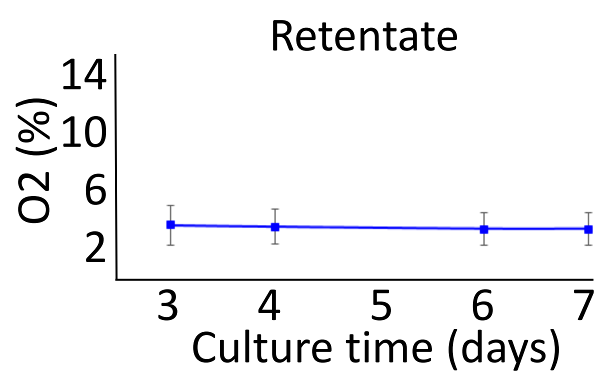






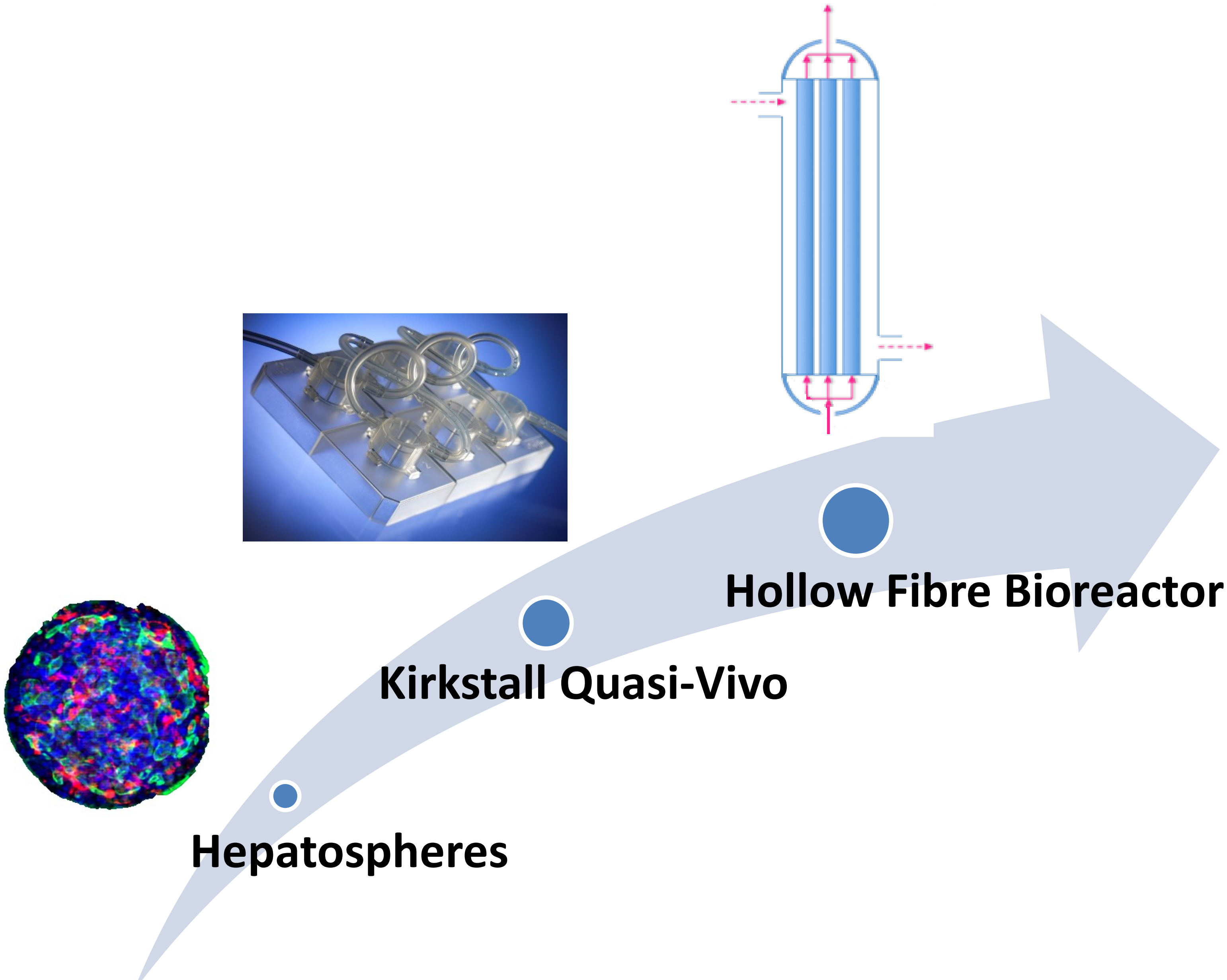
number fibres	3
volumetric flow rate microlitres per hour	400
average single fibre flow (m/s)	1.31E-04
length bioreactor (mm)	60





HFB set-up optimised via mathematical predictions

Mathematical modelling to improve and optimize the design of 3D liver in vitro models



Toxicology Research

ROYAL SOCIETY OF CHEMISTRY

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Characterization of a functional C3A liver sph... model†

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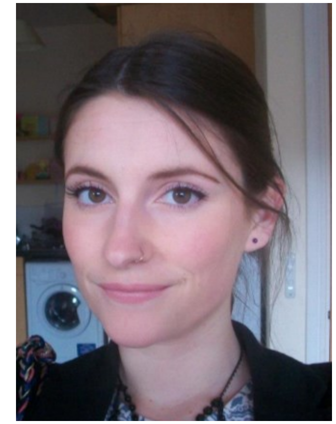
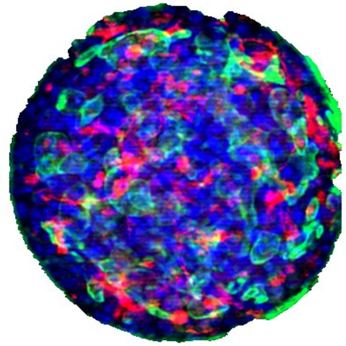
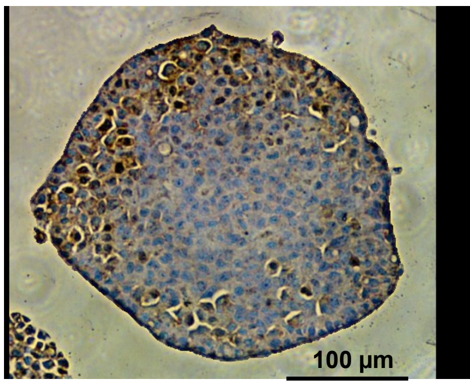
Dominic P. Williams[b] and Steven D. Webb[d]

Harriet Gaskell

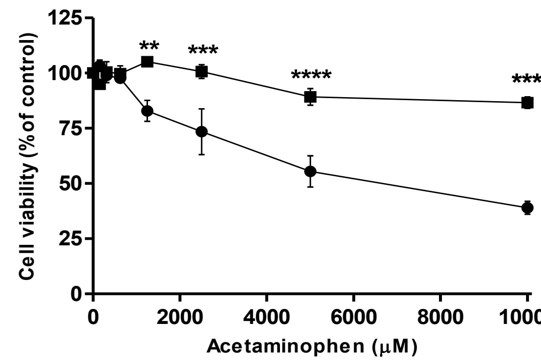
THE UNIVERSITY OF CHICAGO



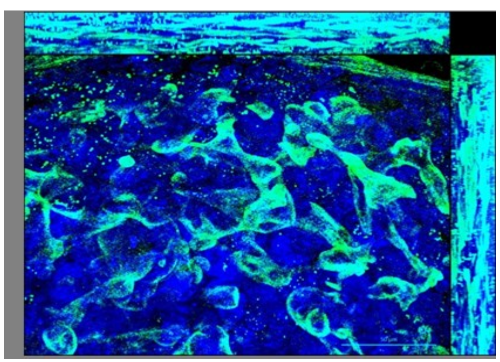




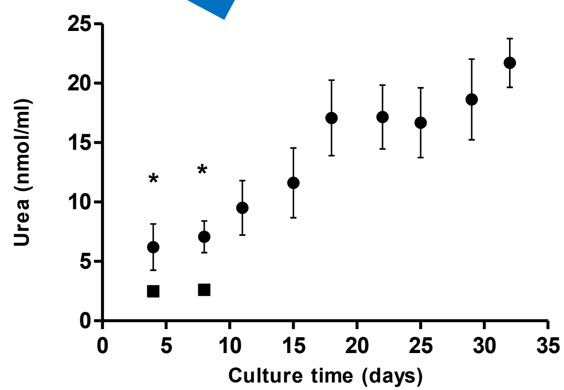
Zonation



Sensitivity to hepatotoxins



Polarisation



Liver-specific function

Gaskell et al, 2016. *Toxicology Research.*

Ingredients for a mathematical model

CRACK IT

Seahorse Technology

- Monitors OCR and ECAR in live cells
- OCR: oxygen consumption rate (*OXPHOS*)
- ECAR: extracellular acidification rate (*glycolysis*)

Maximum O_2 consumption rates:

Fresh human Hep:

$$V_{max} = 7.8 \times 10^{-3}\ mol\ m^{-3} s^{-1}$$

HepG2/C3A:

$$V_{max} = 19.6 \times 10^{-3}\ mol\ m^{-3} s^{-1}$$

Half maximal O_2 concentration:

$$K_m = 6.24 \times 10^{-3} mol\ m^{-3}$$

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Amy Chadwick & Laleh Kamalian

Ingredients for a mathematical model

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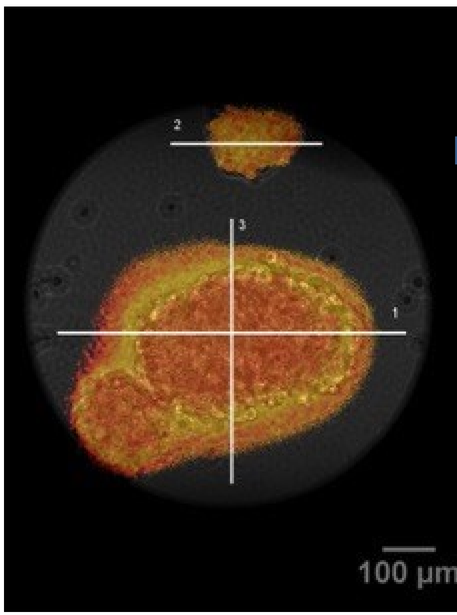
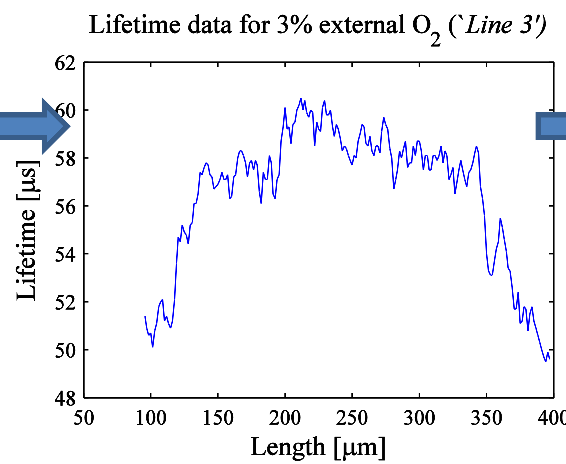
FLIM imaging technique

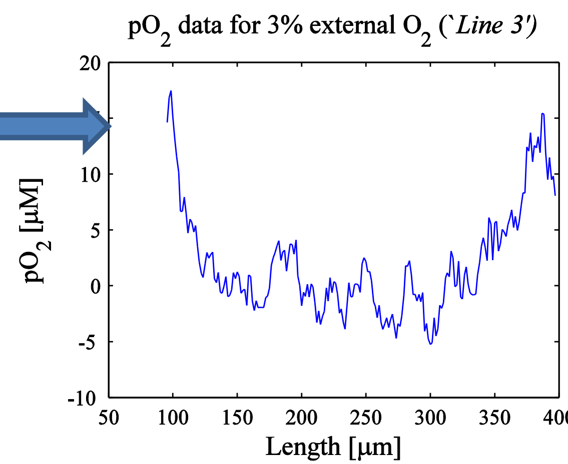
Intracellularly loaded nanoprobes emit phosphorescence that decays in the presence of oxygen.

Greater lifetime value
= lower decay rate
= less oxygen.



HeLa cells





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Leedale, J et al (2014), Math BioSci, 258:33-43

Ingredients for a mathematical model

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Time equilibrium system, spherical coordinates and radial symmetry:

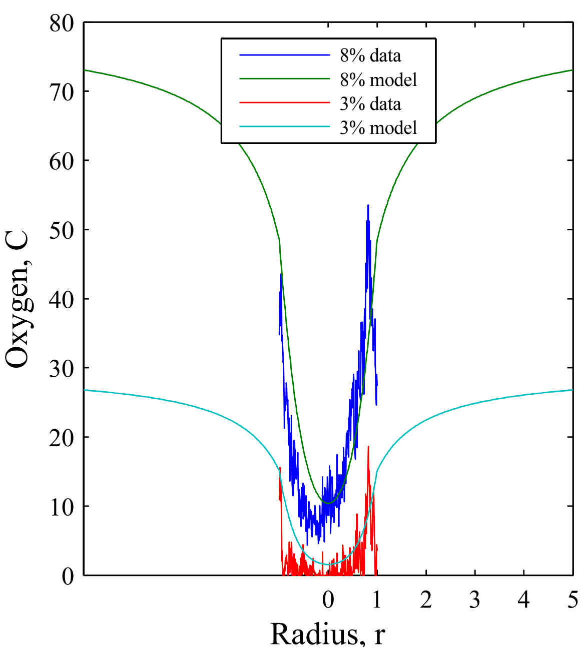
C : Oxygen concentration
D_1, D_2 : Diffusion inside/outside the spheroid
V_{max} : Max consumption rate (constant)
K_m: Half maximal concentration.

INSIDE the Sphere

$$D_1 \nabla^2 C - \frac{V_{max}\, C}{C + K_m} = 0 \qquad r \leq \mathrm{R},$$
$$\frac{\partial C}{\partial r} = 0, \qquad r = 0.$$

OUTSIDE the Sphere

$$D_2 \nabla^2 C = 0 \qquad \infty \geq r \geq \mathrm{R},$$
$$C \to C_\infty, \qquad r \to \infty.$$



$$C_\infty = 3\%\ O_2$$
$$C_\infty = 8\%\ O_2$$

DIFFUSION RATE INSIDE THE SPHEROID:
$$D_1 = 3.84 \text{ to } 4.23 \times 10^4\ \mu\mathrm{m}^2\mathrm{min}^{-1}$$

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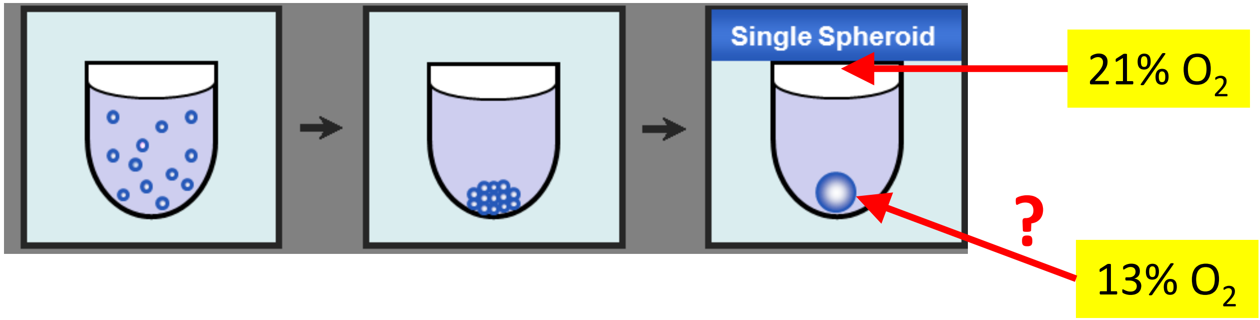
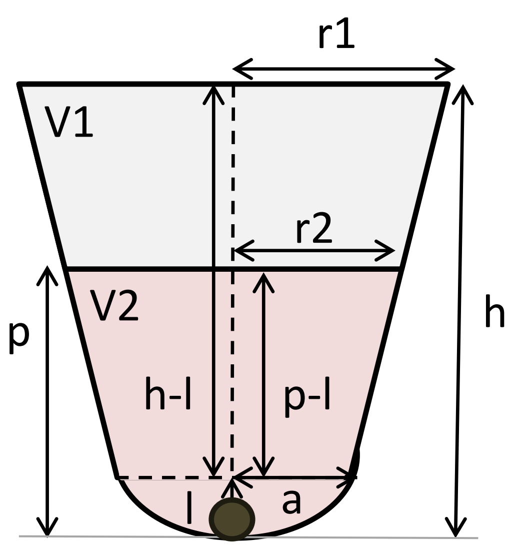
Leedale, J et al (2014), Math BioSci, 258:33-43

Ingredients for a mathematical model

CRACK IT





V1	Working vol per well	360ml
V2	Media vol	100ml
r1	Well radius @ top	3.429mm
h	Depth of well	11.303mm
a	Well radius @ bottom	3.175mm
l	Spherical cap height	1.6mm
r2	Media radius @ top	3.2338mm
p	Media depth	3.848mm

Ingredients for a mathematical model

Time equilibrium system, cylindrical coordinates and radial symmetry, C(r,z):

$$0 = \frac{D_2}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) + D_2\frac{\partial^2 C}{\partial z^2},$$

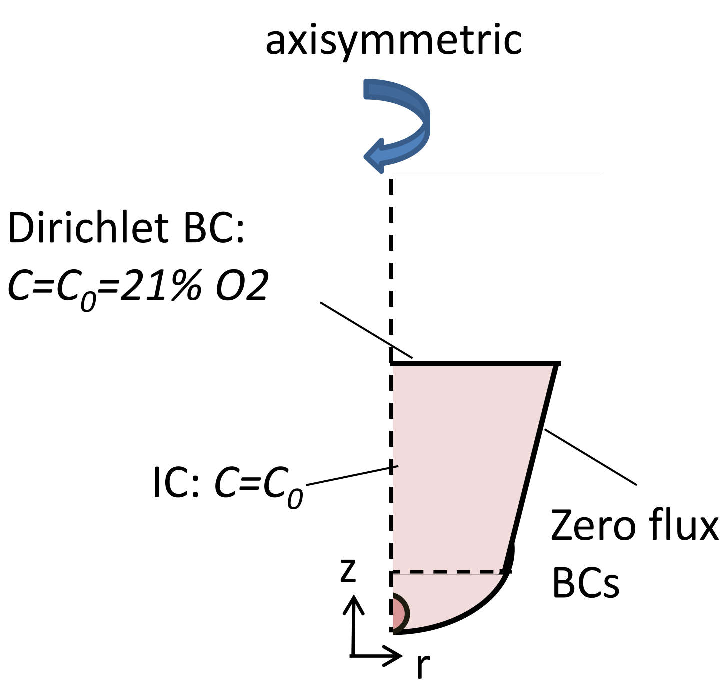
O2 diffusion in media

DIFFUSION RATE OUTSIDE SPHEROID:
$D_2 = 1.15 \text{ to } 1.28 \times 10^5\ \mu\text{m}^2\text{min}^{-1}$

Diffusion rate determined from exp fitting by Joe Leedale in (2014), Math BioSci, 258:33-43

$$0 = \frac{D_1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) + D_1\frac{\partial^2 C}{\partial z^2} - \frac{V_{\max}C}{K_m + C},$$

O2 diffusion and consumption by cells inside spheroid



Model predictions

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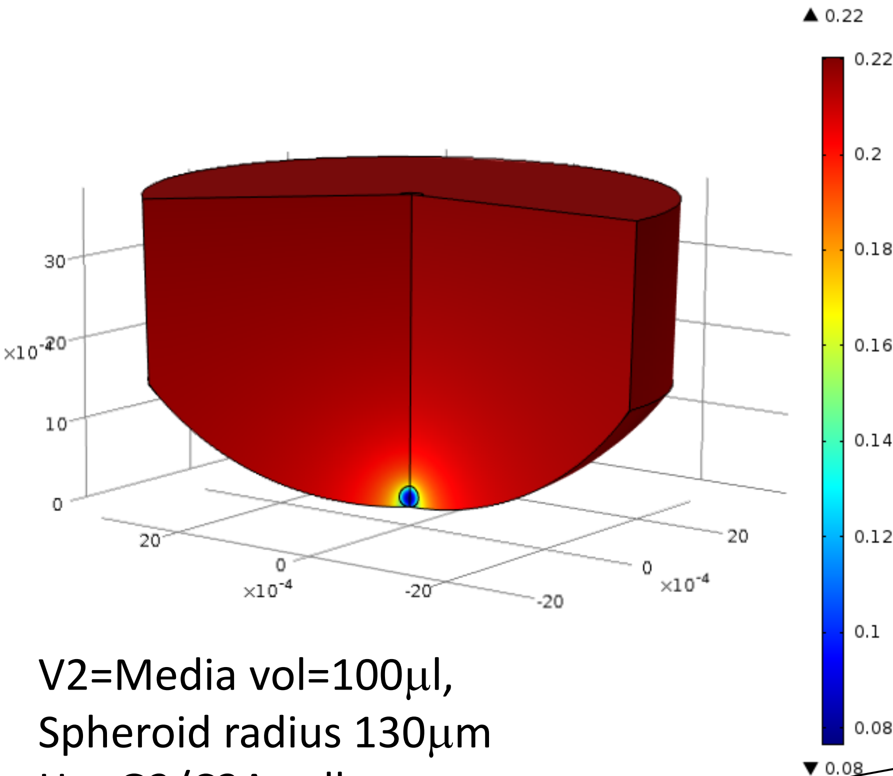
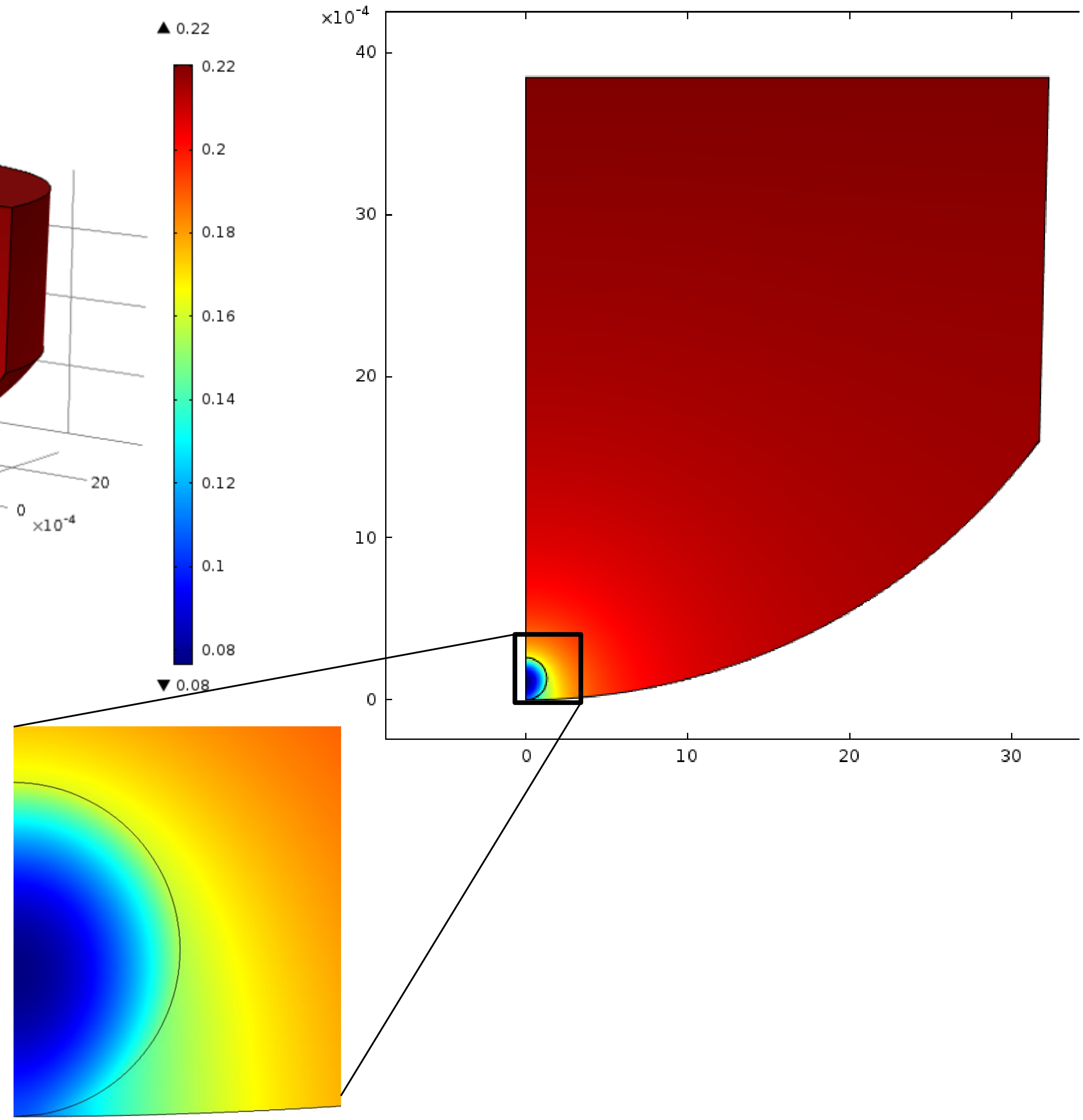
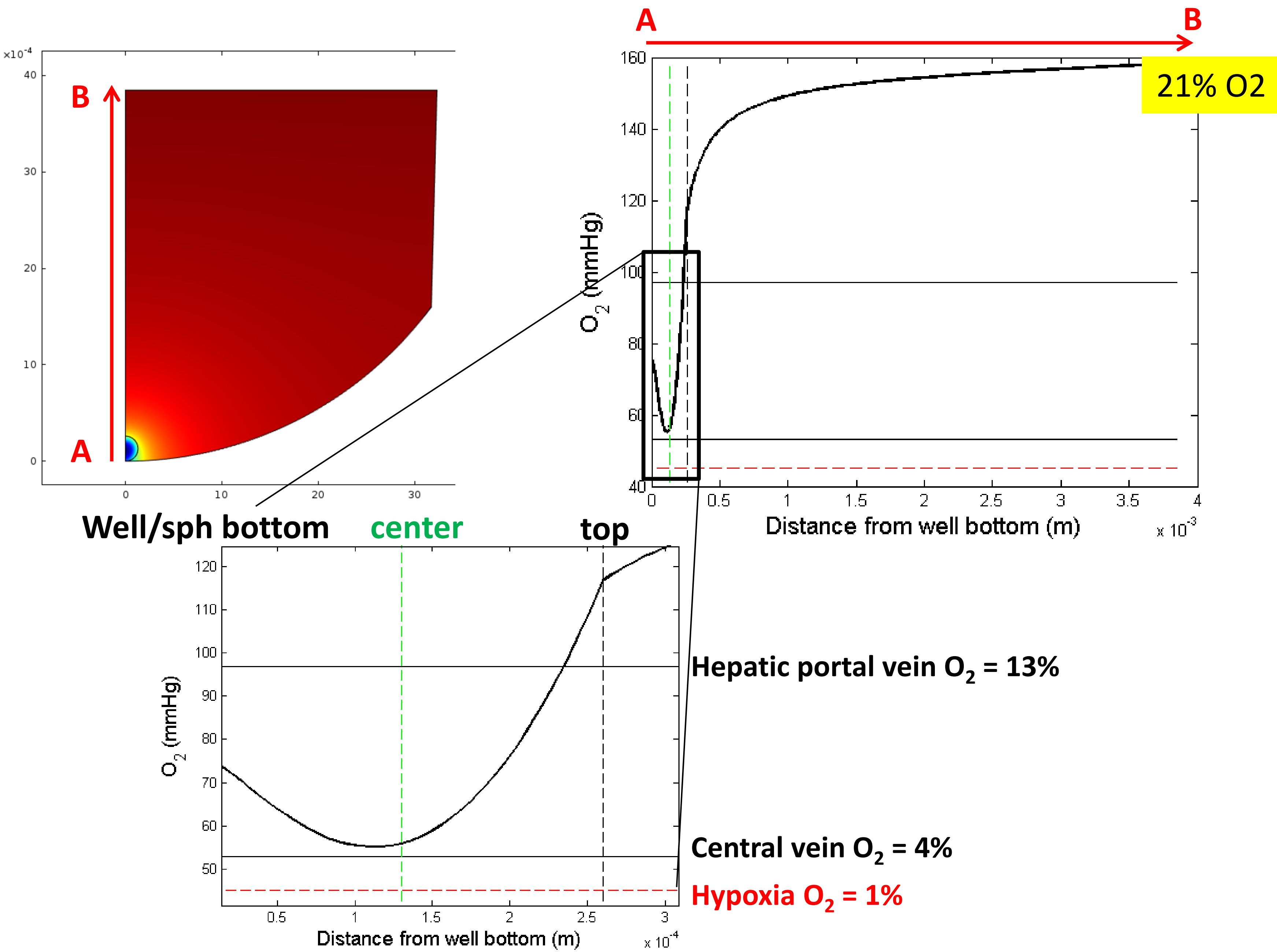


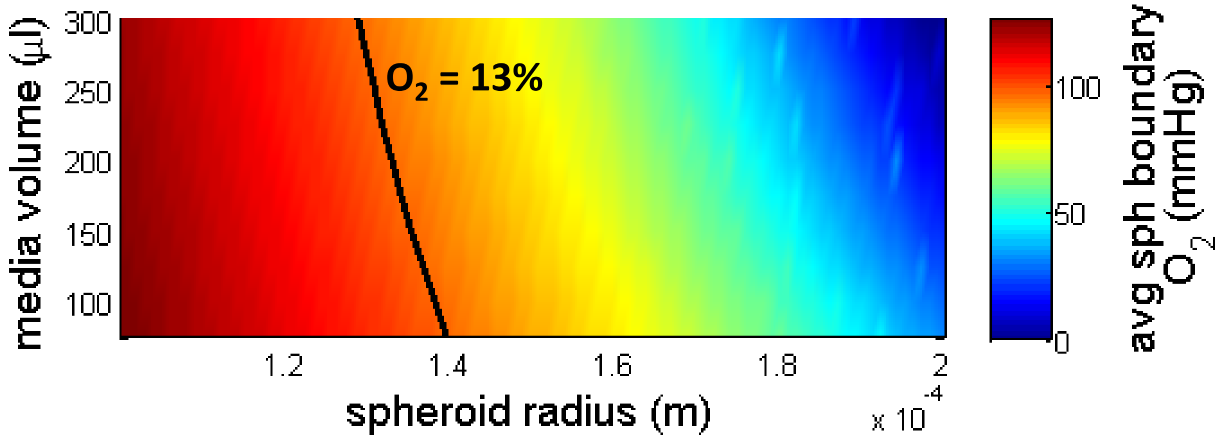
V2=Media vol=100µl,
Spheroid radius 130µm
HepG2/C3A cells

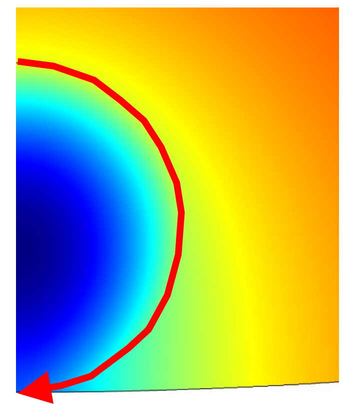


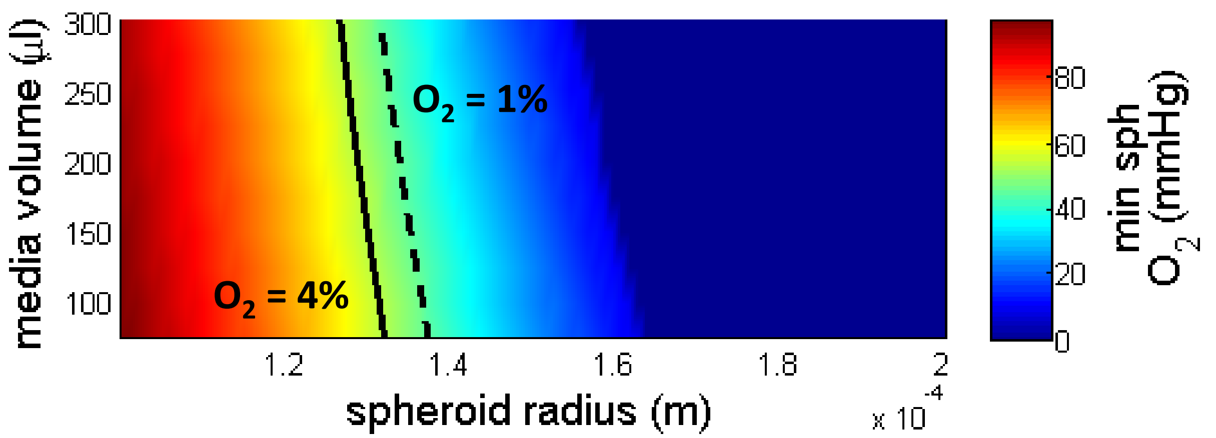
Model predictions

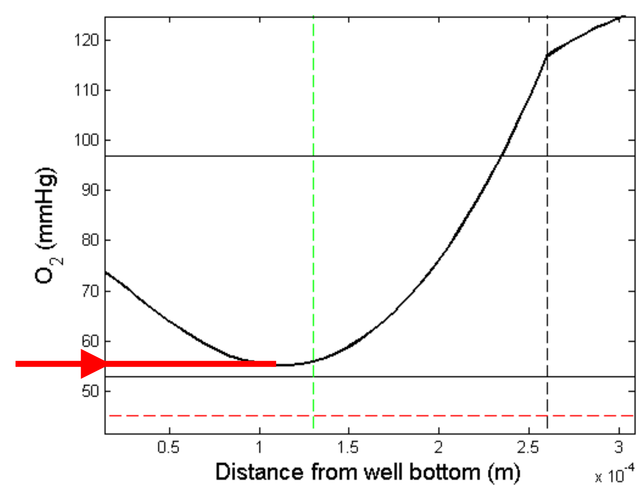


Model predictions: assay sensitivity

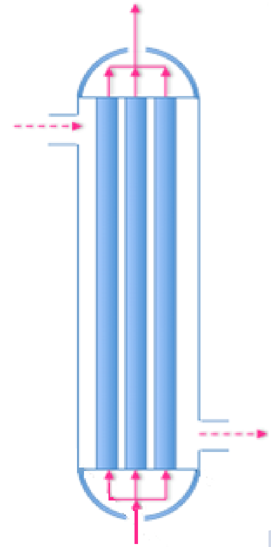






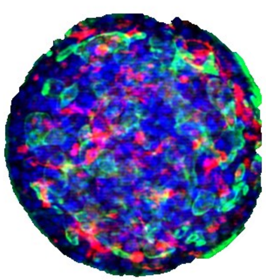


Mathematical modelling to improve and optimize the design of 3D liver in vitro models



Hollow Fibre Bioreactor

Kirkstall Quasi-Vivo



Hepatospheres

Mathematical modelling to improve and optimize the design of 3D liver in vitro models

CRACK IT

Hollow Fibre Bioreactor

Kirkstall Quasi-Vivo

Hepatospheres

Sean McGinty

University of Glasgow

Mathematical modelling of the Kirkstall Quasi-Vivo system: QV900

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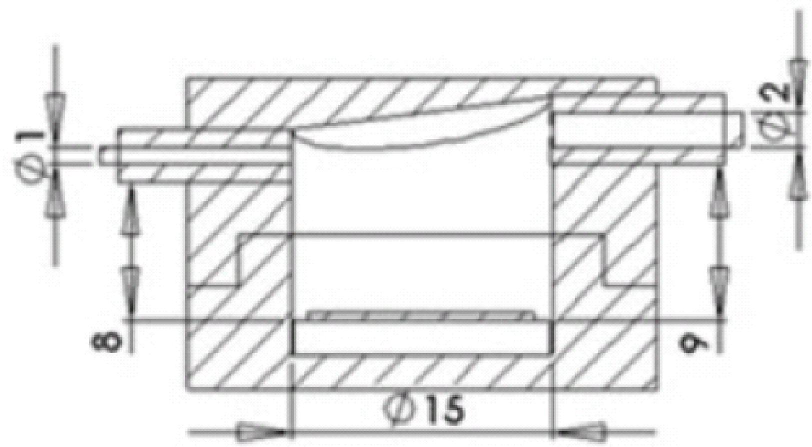


Navier-Stokes equations for the fluid and a convection-diffusion equation for the oxygen concentration:

$$
\begin{aligned}
\nabla.\mathbf{u} &= 0 \\
\rho\frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u}.\nabla)\mathbf{u} &= -\nabla p + \mu\nabla^2\mathbf{u} \\
\frac{\partial c}{\partial t} + (\mathbf{u}.\nabla)c &= D\nabla^2 c
\end{aligned}
$$

u=flow velocity, r= (const) fluid density, p=pressure, m=dynamic viscosity, c=O_2 conc, D=diff coeff in fluid.

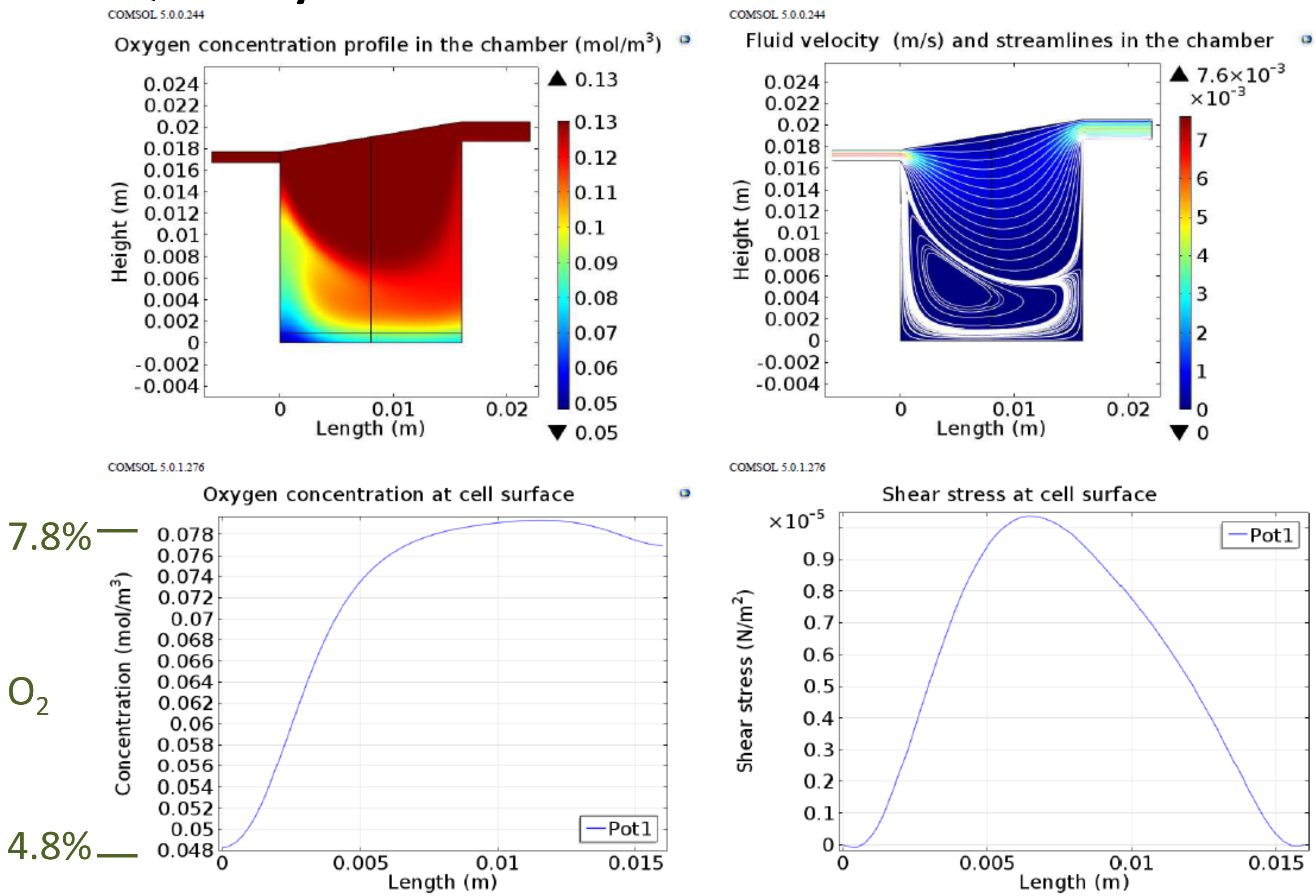
Boundary conditions:
Inlet: constant concentration
Outlet: normal flux
Zero flux conditions everywhere else

A monolayer of of cells is included at the lower boundary. Oxygen obeys a reaction-diffusion model:

$$
\frac{\partial c}{\partial t} = D_g\nabla^2 c - \frac{V_{\max} c}{K_m + c}
$$

V_{max}= max cell O_2 consumption rate, K_m=half maximal O_2 conc, D_g=diff coeff in cell layer.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system



Result for Q = 180uL/min. **Top left:** O2 concentration profile. **Top right:** Flow profile. **Bottom left:** O2 concentration at the cell surface. **Bottom right:** Shear stress at the cell surface.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

CRACK IT

- **Question:** can we capture an O2 range of 13%-4% at the cell layer in a single pot?

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

CRACK IT

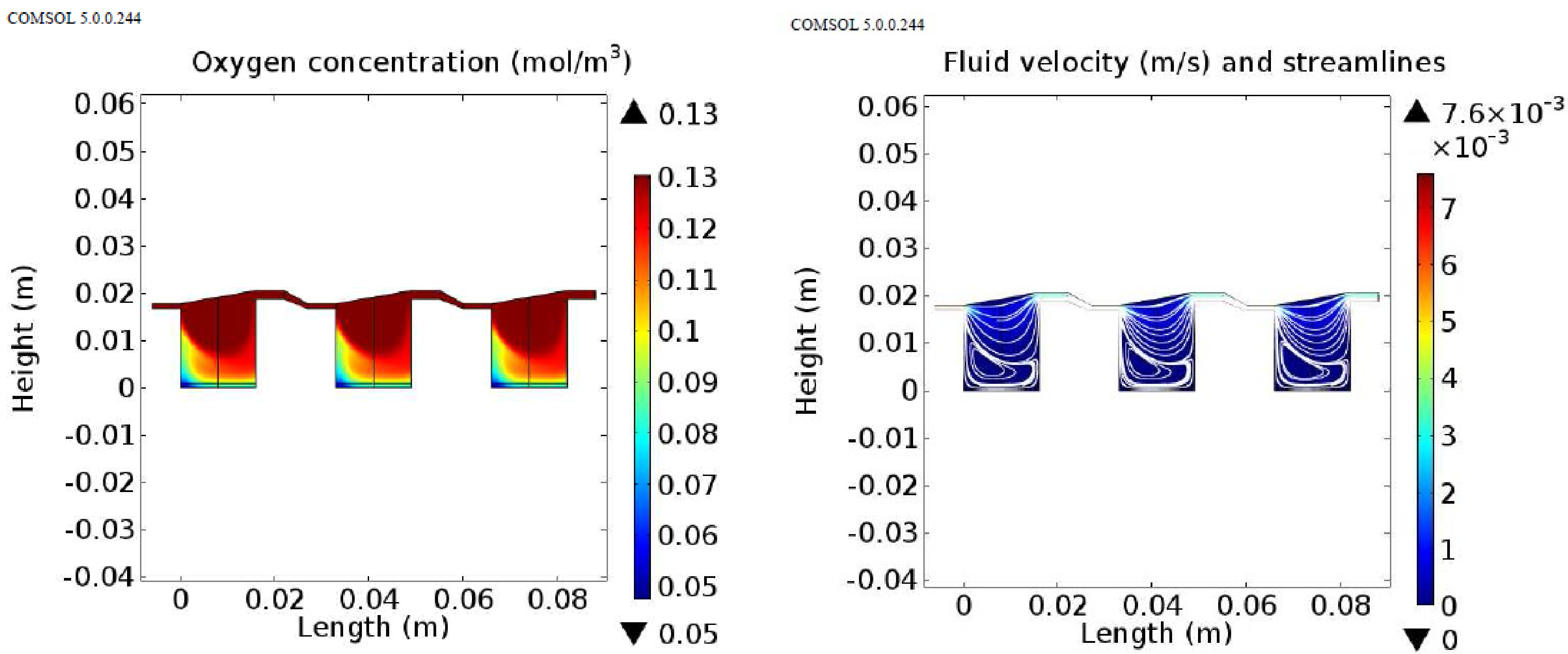
- **Question:** can we capture an O2 range of 13%-4% at the cell layer in a single pot?
  - Explored flow rates ranging from 60-1000ul/min.
  - Gives O2 ranges from 3.9%-6.9% to 10.4%-12.2%.
  - Shear stress ranges from 1e-5 to 7e-4 N/m^2
  - Increasing height of cells in pots increases min & max O2 as well as shear stress.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

- **Question:** can we capture an O2 range of 13%-4% at the cell layer in a single pot?

- **Question:** can we capture an O2 range of 13%-4% at the cell layer across multiple pots?

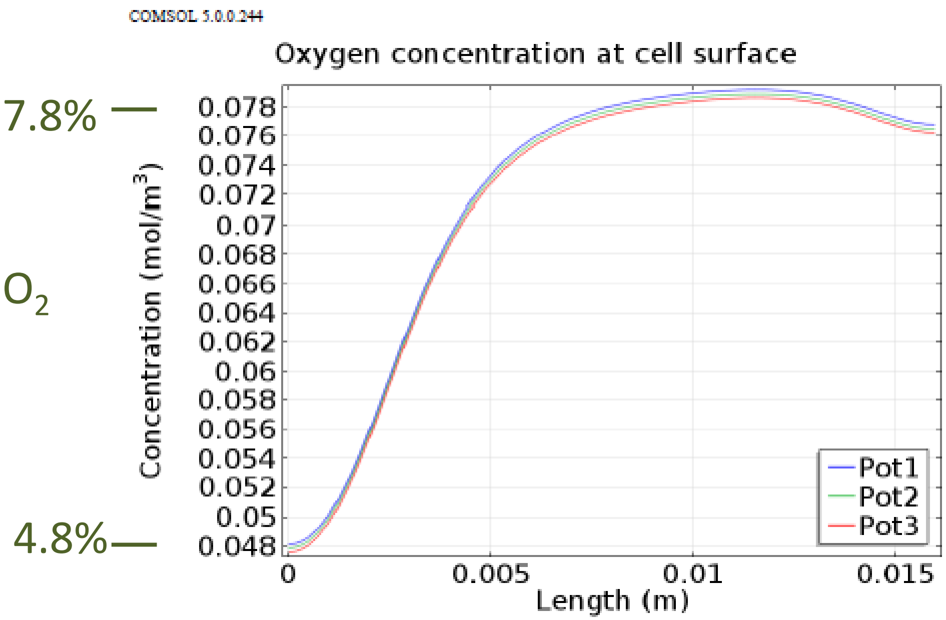
Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

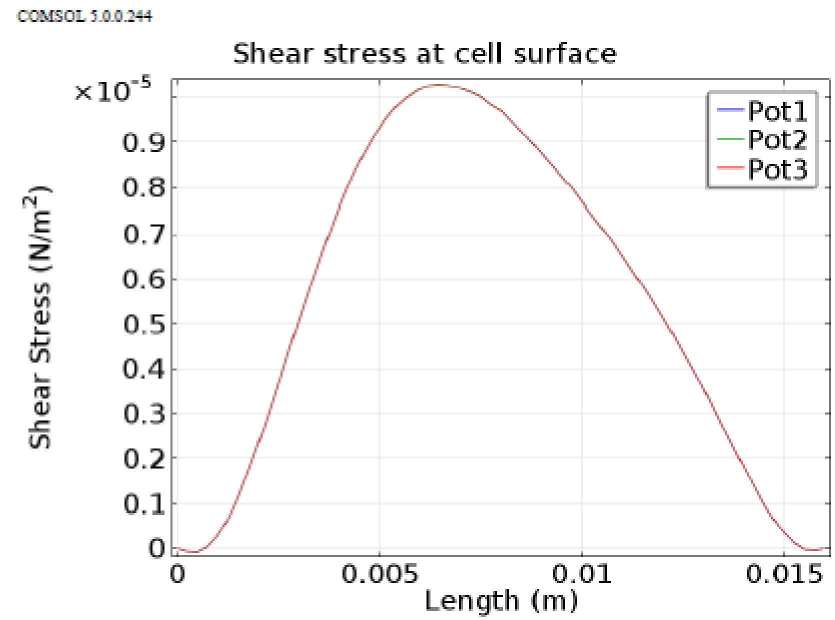


Result for Q = 180uL/min. **Left:** O2 concentration profile. **Right:** Flow profile.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

CRACK IT





Result for Q = 180uL/min. **Left:** O2 concentration at the cell surface. **Right:** Shear stress at the cell surface.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

CRACK IT

- **Question:** can we capture an O_2 range of 13%-4% at the cell layer in a single pot?

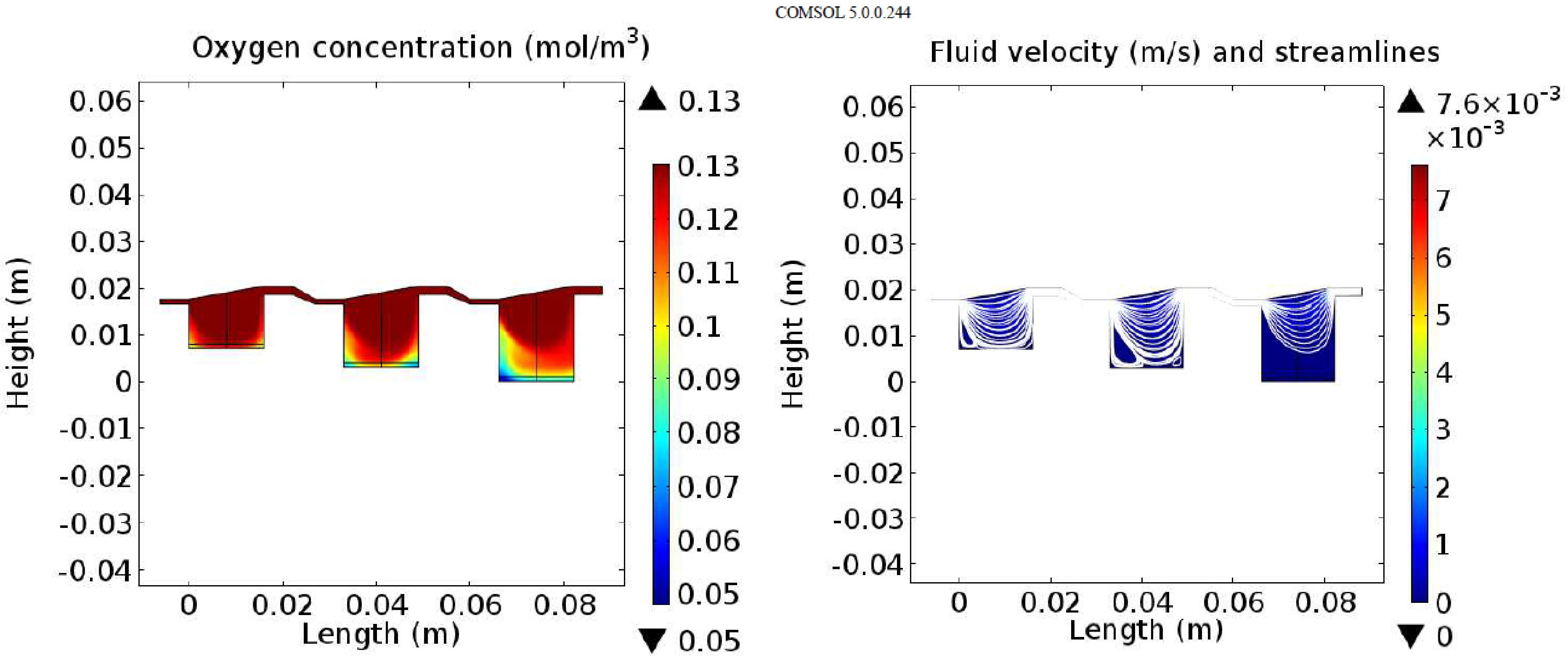
- **Question:** can we capture an O_2 range of 13%-4% at the cell layer across multiple pots?
    - O_2 profiles, shear stress very similar in each chamber.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

CRACK IT

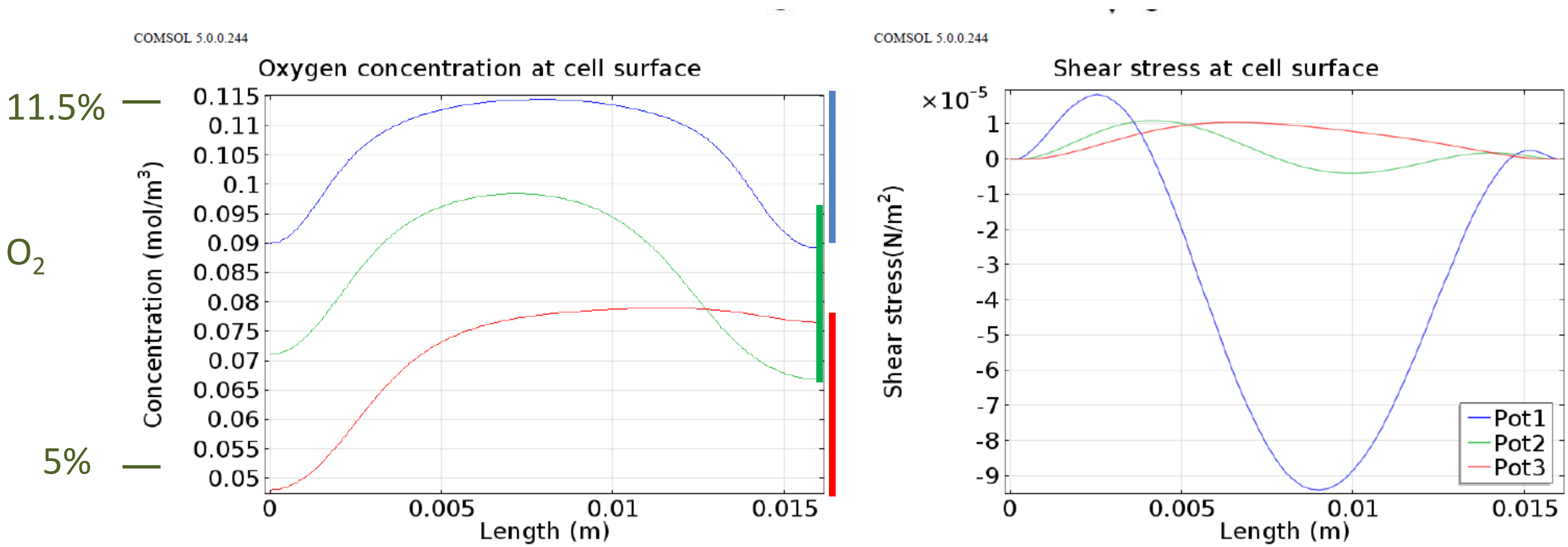
- **Question:** can we capture an O2 range of 13%-4% at the cell layer in a single pot? ✗
- **Question:** can we capture an O2 range of 13%-4% at the cell layer across multiple pots? ✗
- **Question:** can we exploit the vertical O2 gradient in the pots?

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system



Result for Q = 180uL/min. Cell surface raised by 7mm, 3mm and 1mm in pots. **Left:** O2 concentration profile. **Right:** Flow profile.

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system



Result for Q = 180uL/min. Cell surface raised by 7mm, 3mm and 1mm in pots. **Left:** O2 concentration at the cell surface. **Right:** Shear stress at the cell surface.

O2 ranges: pot 1, 9%-11.5%; pot 2, 7%-10%; pot 3, 5%-7.7%

Mathematical modelling of the Kirkstall Quasi-Vivo QV900 system

- **Question:** can we capture an O_2 range of 13%-4% at the cell layer in a single pot?

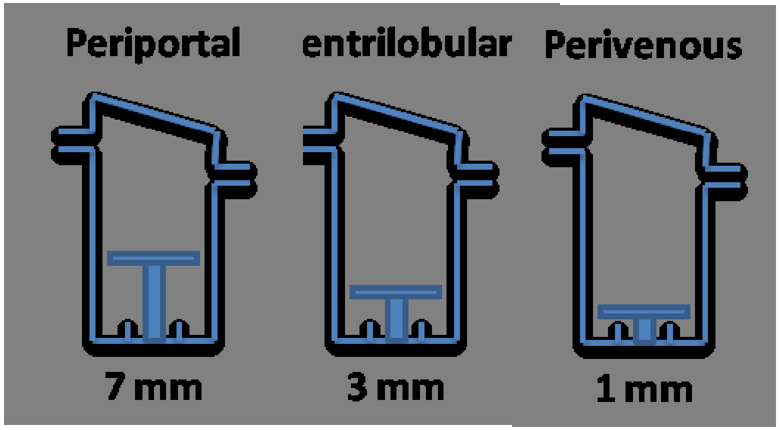
- **Question:** can we capture an O_2 range of 13%-4% at the cell layer across multiple pots?

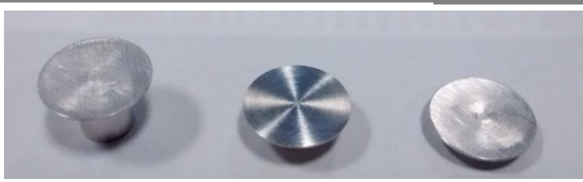
- **Question:** can we exploit the vertical O_2 gradient in the pots?

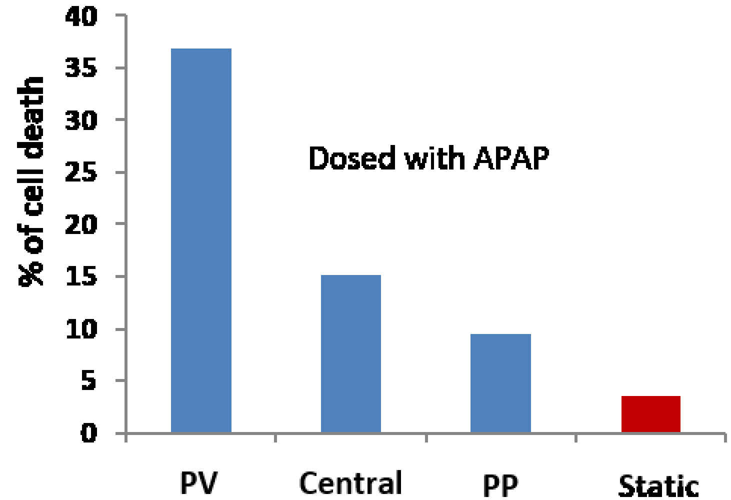
    - **O_2 ranges:** pot 1, 9%-11.5%; pot 2, 7%-10%; pot 3, 5%-7.7%.

Kirkstall QV – Zonated Liver Adaptation

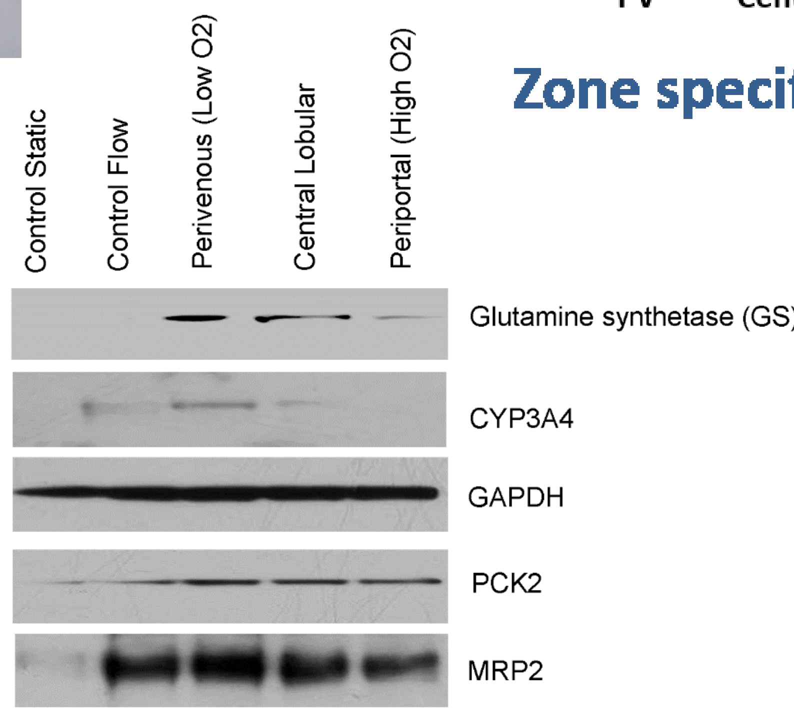
CRACK IT





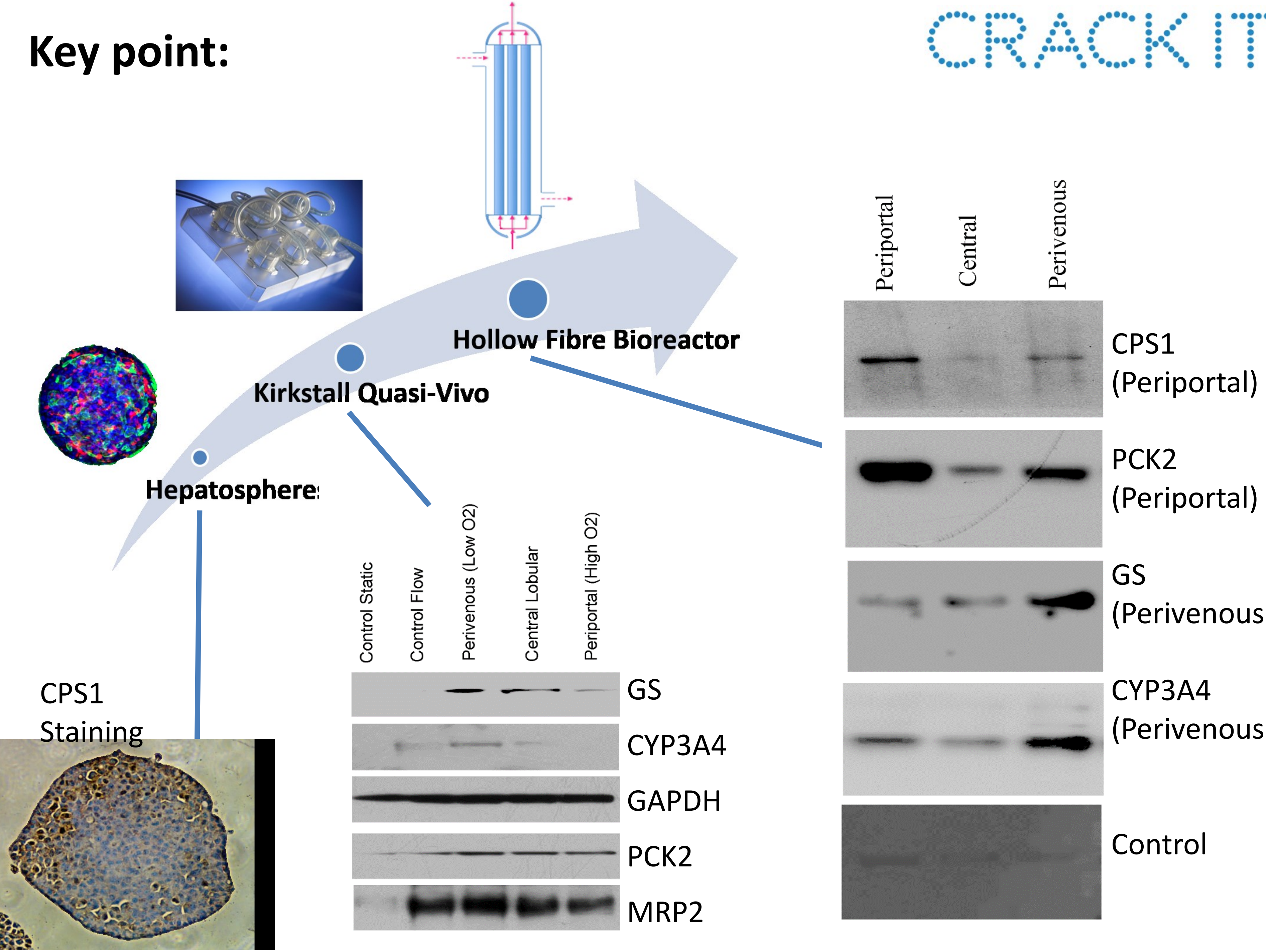


Zone specific metabolism



Expression of "zonation marker" proteins, following 6 days growth. Static control included.

Key point:


Hollow Fibre Bioreactor
Kirkstall Quasi-Vivo
Hepatospheres
CPS1
Staining
Control Static
Control Flow
Perivenous (Low O2)
Central Lobular
Periportal (High O2)
GS
CYP3A4
GAPDH
PCK2
MRP2
Periportal
Central
Perivenous
CPS1
(Periportal)
PCK2
(Periportal)
GS
(Perivenous)
CYP3A4
(Perivenous)
Control

Many thanks to...

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NC3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

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University of Bath

Kim Leutchford

University of Bath

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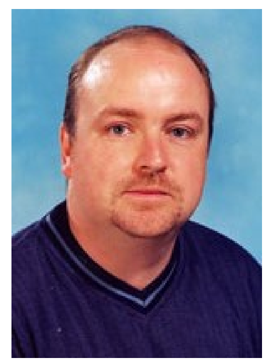
UCL



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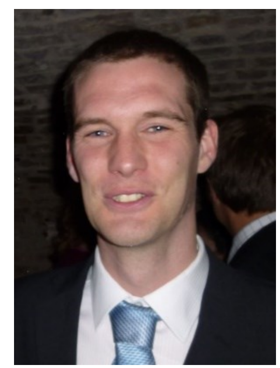

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