multiD in-vitro models

“The stress of shear"

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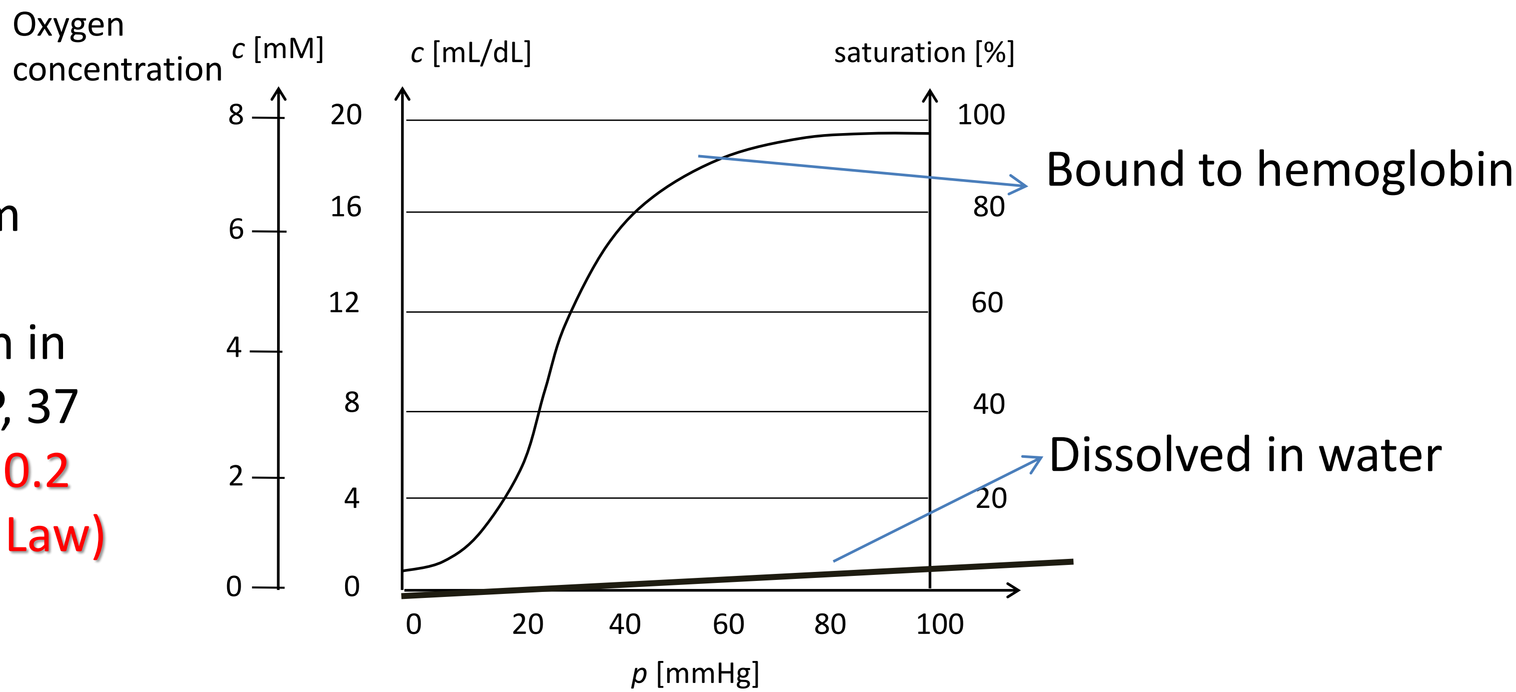
Why is oxygen the problem *in vitro*?

The maximum oxgyen concentration in water (@ BTP, 37 °C, 1 atms) is 0.2 mM (Henry’s Law)



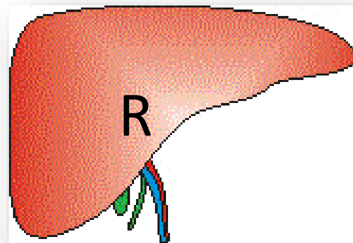
Typical concentrations

	Blood	Interstitial fluid
Oxygen	5-8 mM	<0.2 mM
Glucose	4-7 mM	2-7 mM

Estimating oxygen consumption rates *in vivo*

Blood flow in, Q_{in}

$$R = Q_{in}C_{in} - Q_{out}C_{out}$$



Blood flow out, Q_{out}

C_{in}

C_{out}

Consumption is highly dependent on organ/tissue function and total number of cells or cell density (usually Michaeles Menten type)

R=Consumption rate (moles/s)

R_c=specific consumption (moles.s^{-1}/cell)

R_{vol}= volumetric consumption rate (moles.m^{-3}. s^{-1})

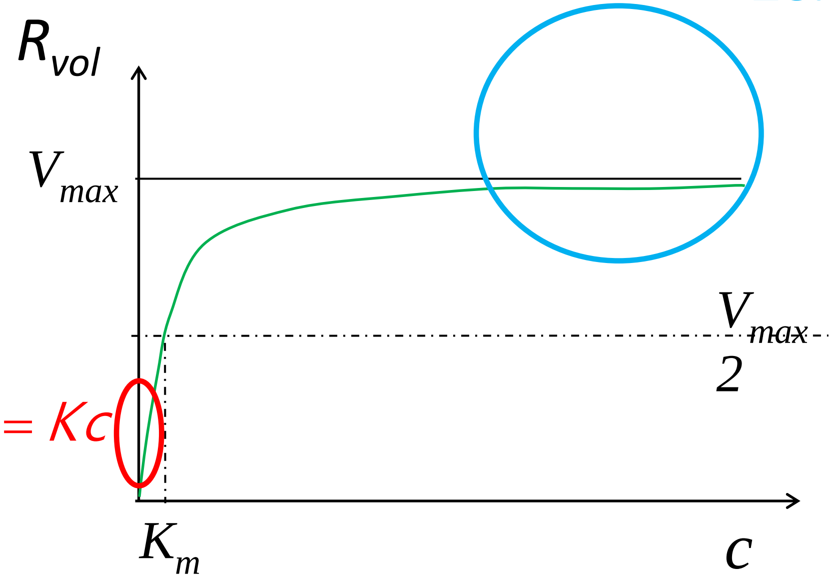
$R_{vol}=R_c$*cell density

Michaelis Menten

$$R_{vol}(c) = V_{max} \cdot \frac{c}{K_m + c}$$

Zero order

$$R_{vol} = V_{max}$$

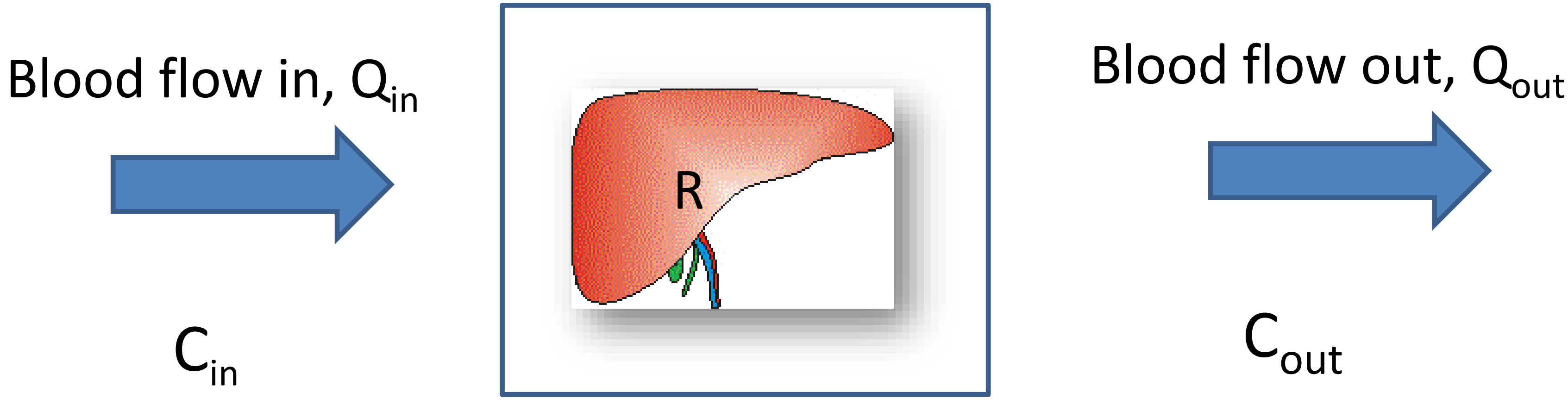


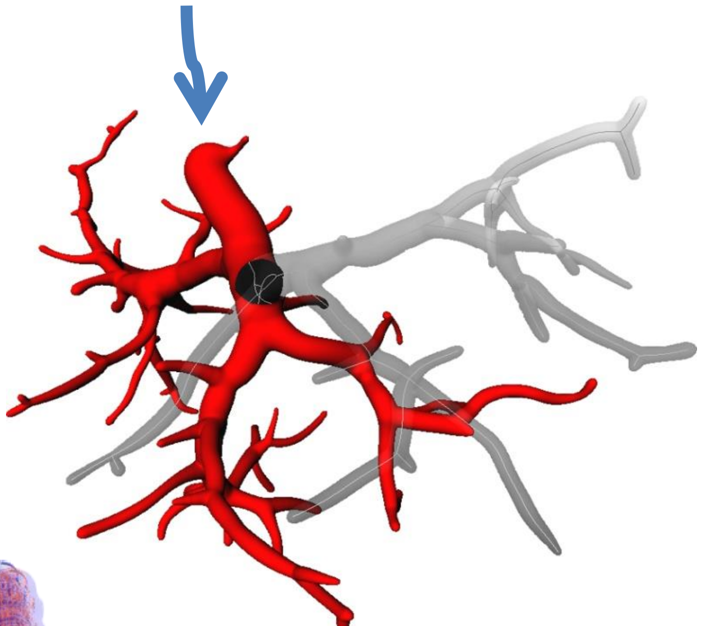
First order

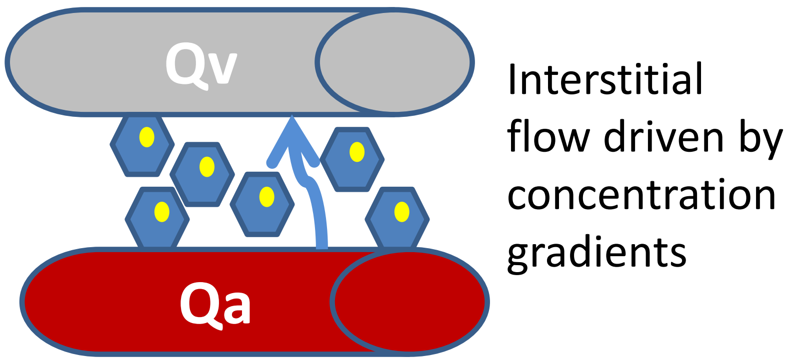
$$R_{vol}(c) = V_{max} \cdot \frac{c}{K_m} = Kc$$

Oxygen consumption rates

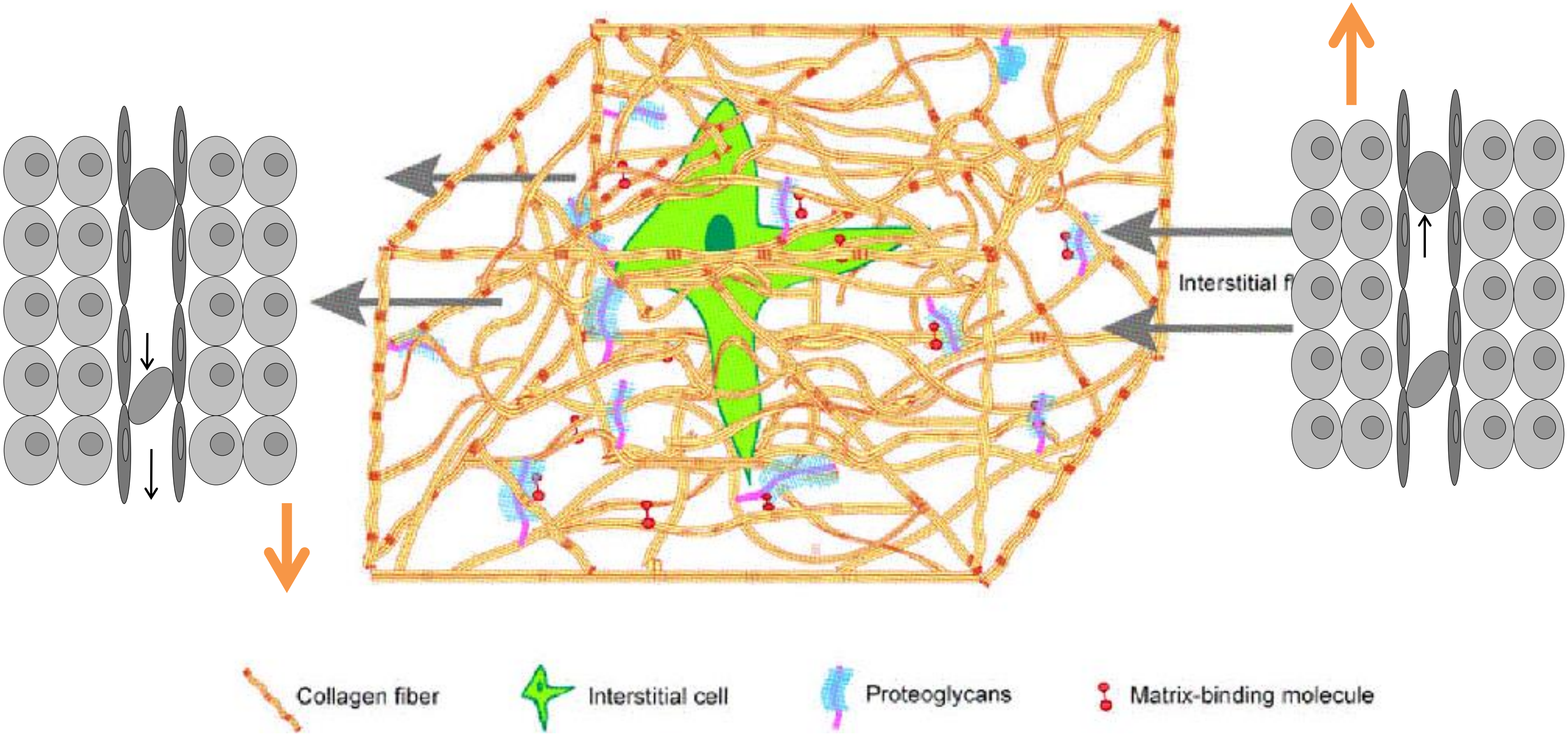
Organ/tissue	R	Rs (moles.s^{-1}/cell)
Whole body	260 mL O_2/min $\rightarrow$ (5×10^{13} cells)	3×10^{-17}
Liver	58 mL O_2/min$\rightarrow$ (2×10^{11} hepatocytes)	3×10^{-16}
Cartilage		3×10^{-19}
Bone marrow SC		1.5×10^{-17}


Blood flow in, Q_{in}
C_{in}
R
Blood flow out, Q_{out}
C_{out}




Qv
Qa
Interstitial flow driven by concentration gradients

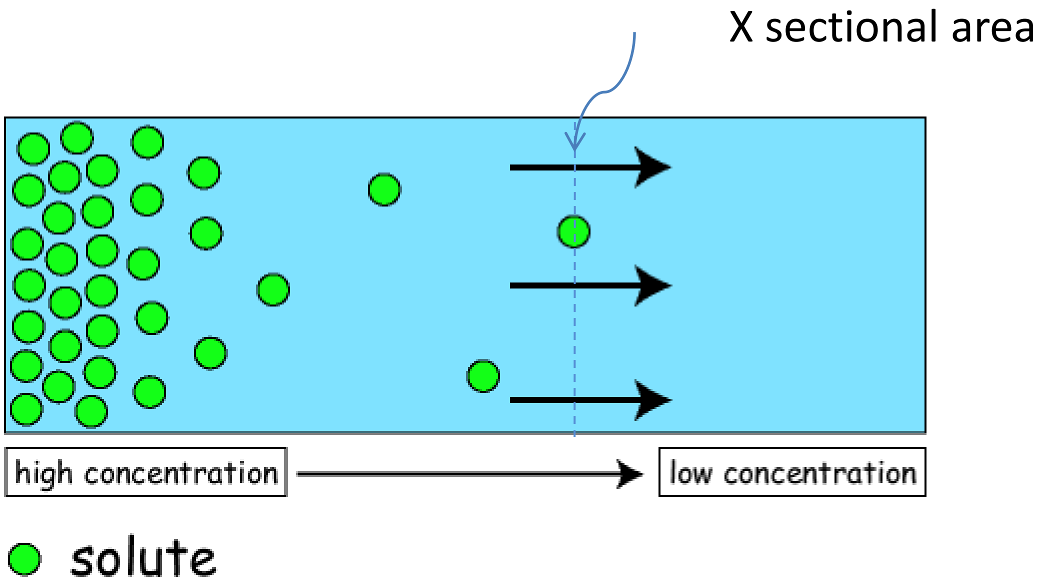
INTERSTITIAL FLOW



1) interstitial flow is due to a concentration gradient 2) all tissues are permeated by interstitial flow 3) the flow is through a microporous medium

Swartz & Fleury, ARBE
Vol. 9: 229-256.2007

Diffusion

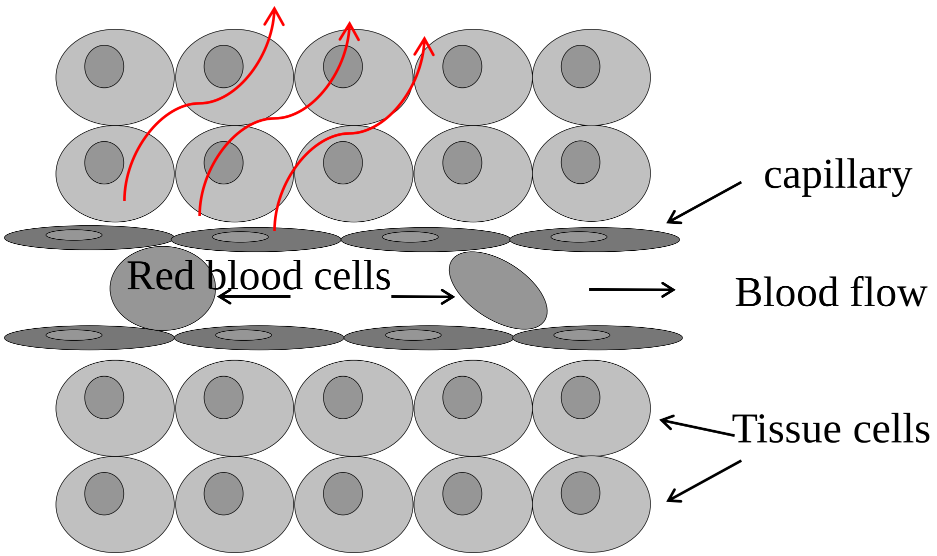


Solute transport is due to the concentration gradient dc/dx.
J is molecular flux rate across unit surface area (moles/m^2/s).
D is the diffusion constant (m^2/s)

$$J = -D\frac{\partial c}{\partial x}, \qquad \frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

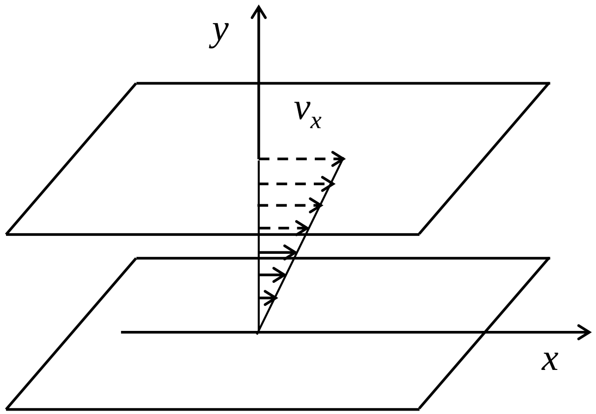
FLOW and SHEAR

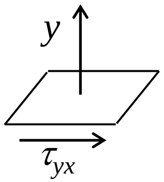
Only epithelial cells (skin, blood vessels, intestine) and the non adherent cells of the immune system and blood can support direct fluid flow.



The motion of fluid across a mobile or semi mobile surface gives rise to **shear stress**

Shear stress





$$\tau_{yx} = -\mu \frac{dv_x}{dy}$$

The shear stress on a monolayer of cells in a flat chamber with flow Q is

$$\tau_{yx} = -\frac{6Q\mu}{wh^2}$$

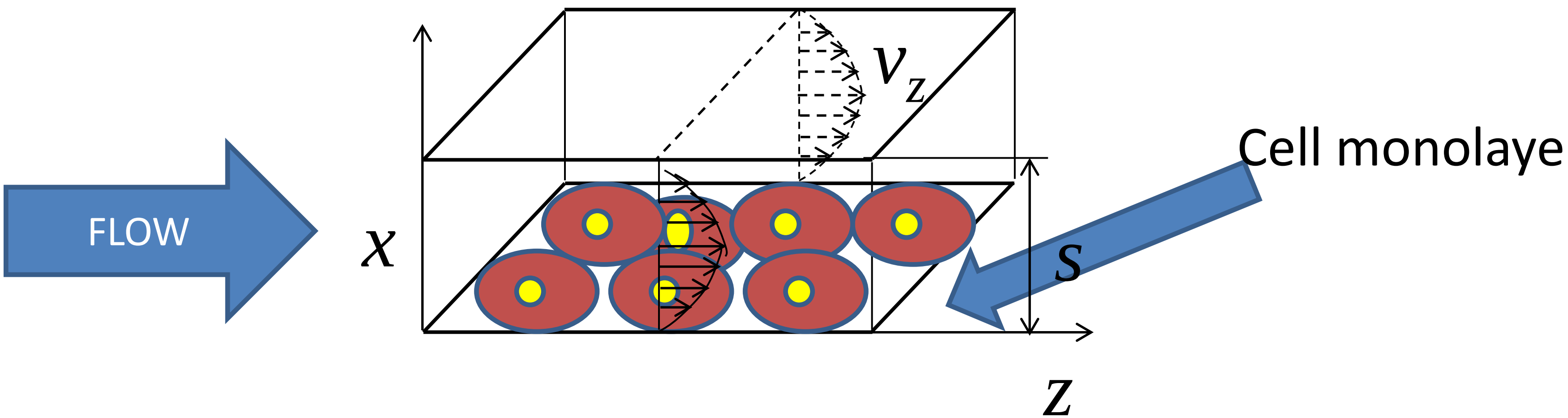
Optimal shear stress in bioreactors

Cell	Shear	Flow rate	Ref
Human trabecular bone, 3D	5.10^{-5} Pa	0.01 mL/min	Porter. Journal of Biomechanics, 38, 543, 2005
Human osteosarcoma cells, 3D	0-0.021 Pa	Max. 25 mL/min	Laganà.Biomedical Microdevices, **14(1), 225, 2012**
hBMSC, 3D	0.015 Pa	3 mL/min	Li. Tissue Eng. A, **15, 2773,2009**
HepG2, 2D	0.14 Pa	0.0025 mL/min	Tanaka et al, Meas. Sci. Technol. **17 ,3167–3170, 2006**
Human hepatocytes, 2D+ gel	5.10^{-5}Pa	0.25 mL/min	Vinci et al. Biotech J., 6(5):554, 2011
Rat hepatocytes, 2D+ fibroblasts	0.014 Pa	0.06 mL/min	Tilles et al, Biotech & Boeng. 73 (5),379 ,2001

Wall shear stress in blood vessels: 1-0.01 N/m^2
For all other (non epithelial) tissues shear is much less (0.01-0.00001 N/m^2), and is due to interstitial flow (few microL/min).

Adding flow

$$\frac{\partial c}{\partial t} = D\nabla^2 c - R_{vol}(c) - v.\nabla c$$



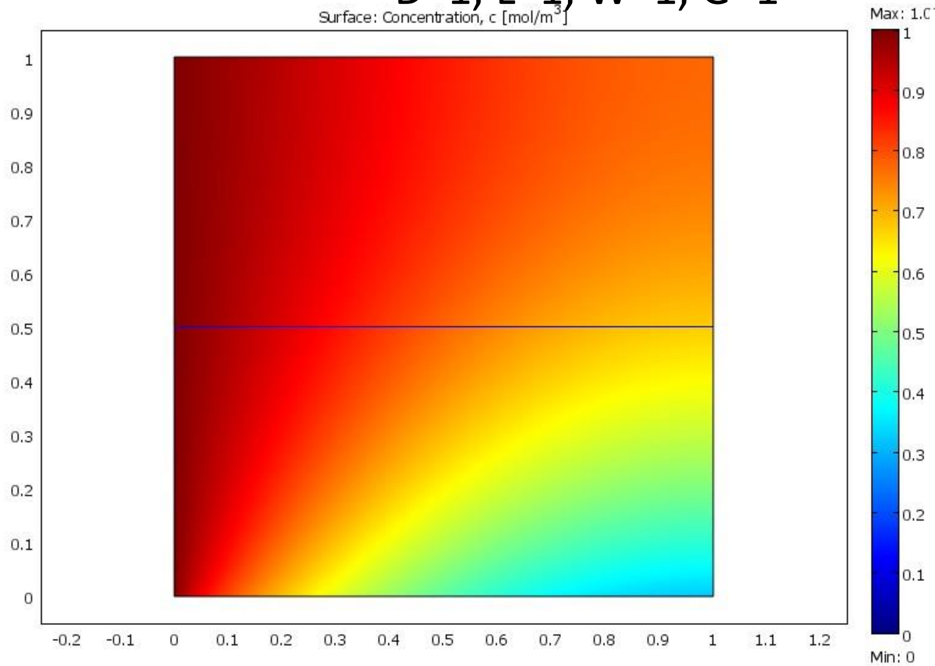
For a monolayer

$$\frac{\partial c}{\partial t} = D\frac{\partial c^2}{\partial x^2} - v_z\frac{\partial c}{\partial z}$$

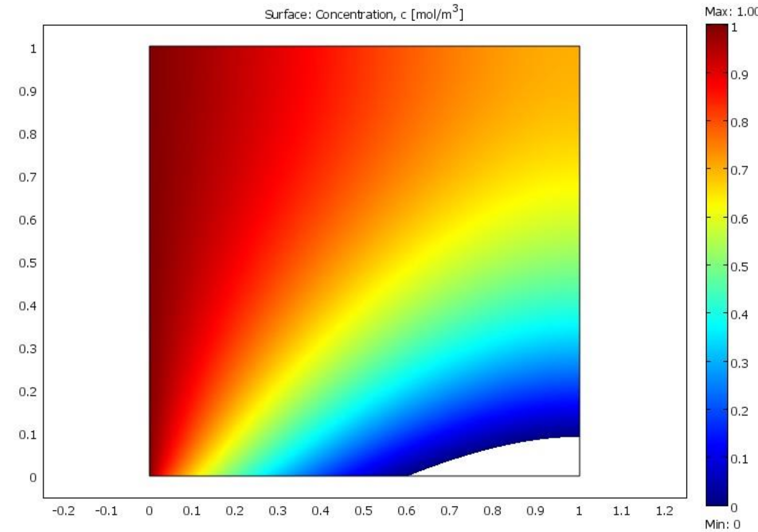
For volumetric consumption

$$\frac{\partial c}{\partial t} = D\frac{\partial c^2}{\partial x^2} - v_z\frac{\partial c}{\partial z} - R_{vol}$$

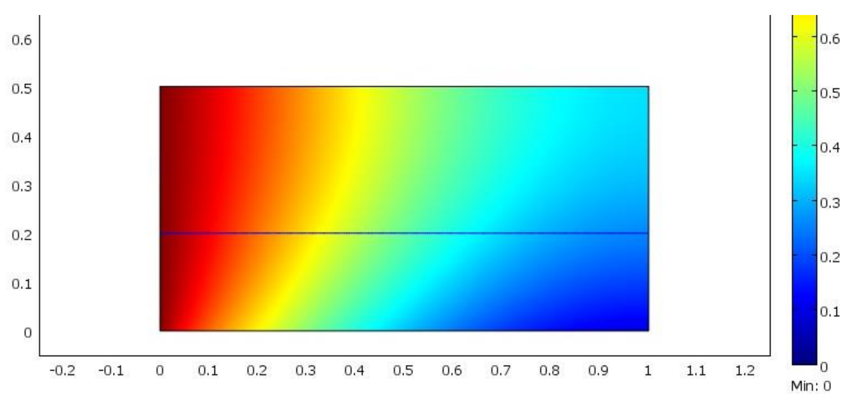
D=1, L=1, W=1, G=1



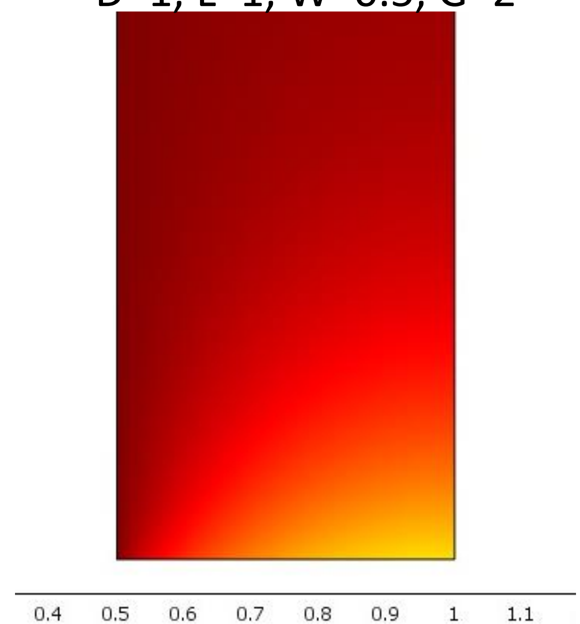
D=2, L=1, W=1, G=0.5

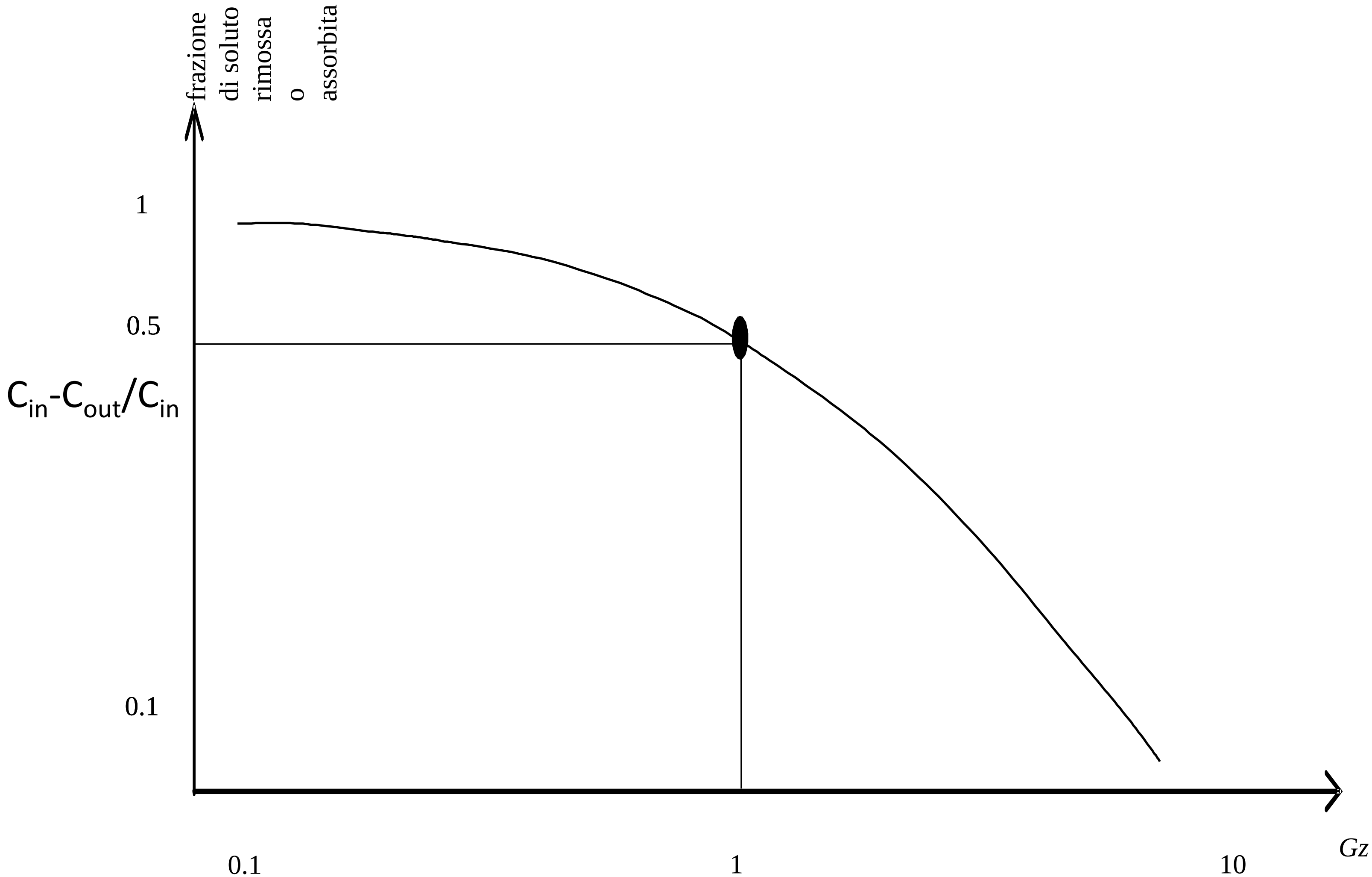


D=1, L=0.5, W=1, G=0.25



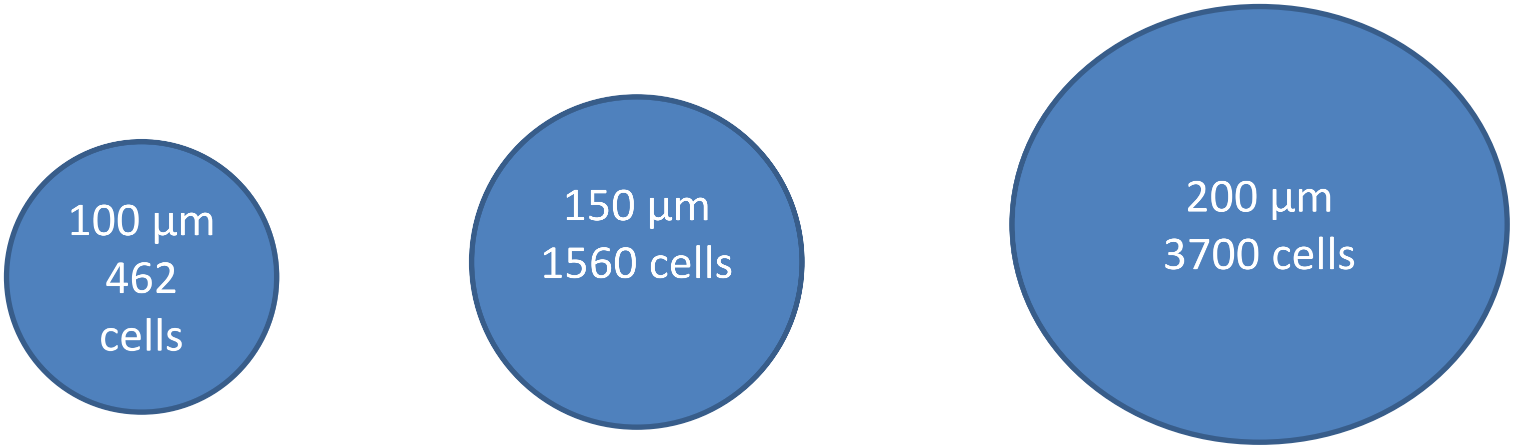
D=1, L=1, W=0.5, G=2





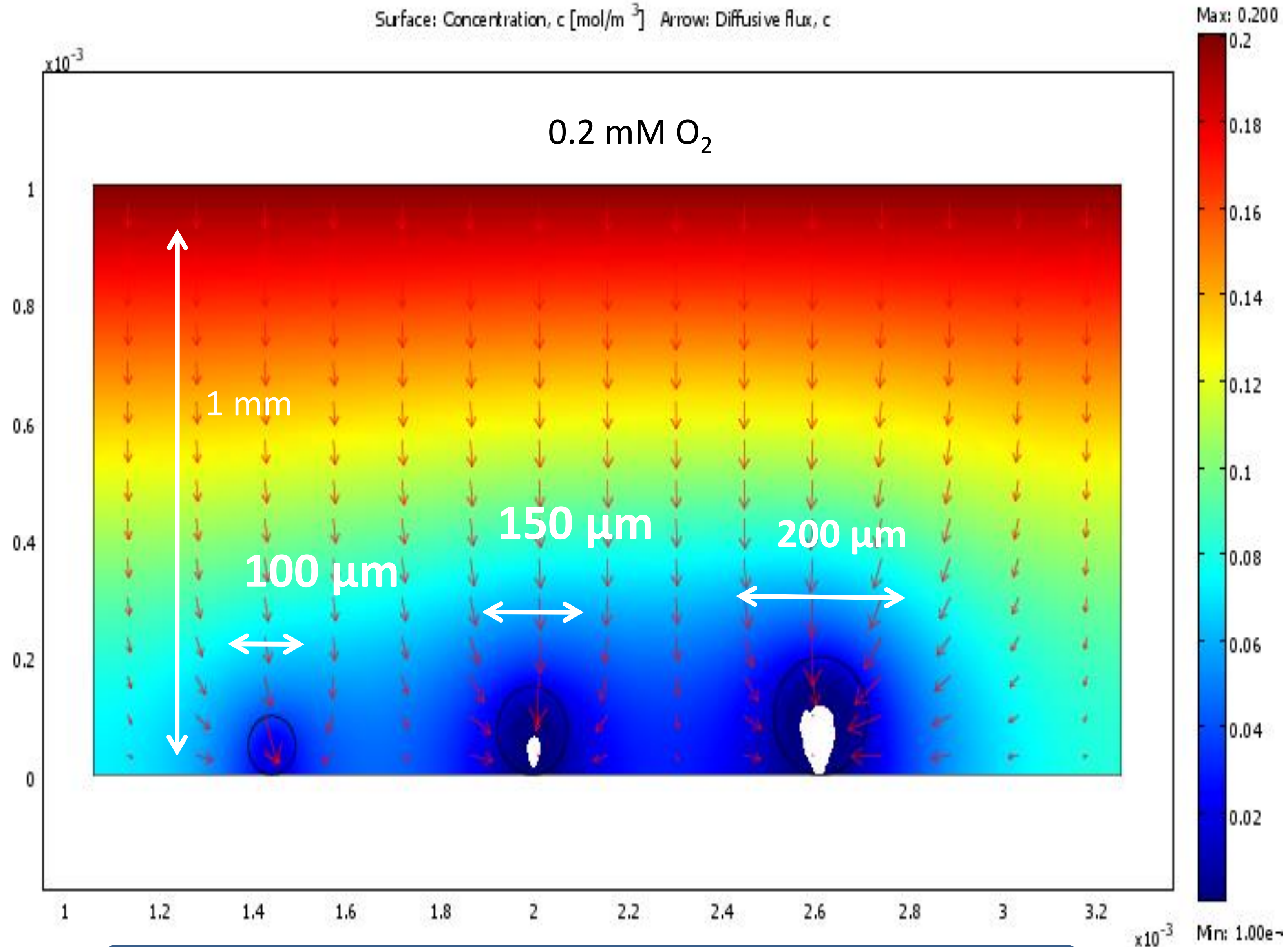
frazione di soluto rimossa o assorbita
1
0.5
C_{in}-C_{out}/C_{in}
0.1
0.1
1
10
Gz

Example 1: Oxygen diffusion in gel encapsulated islets



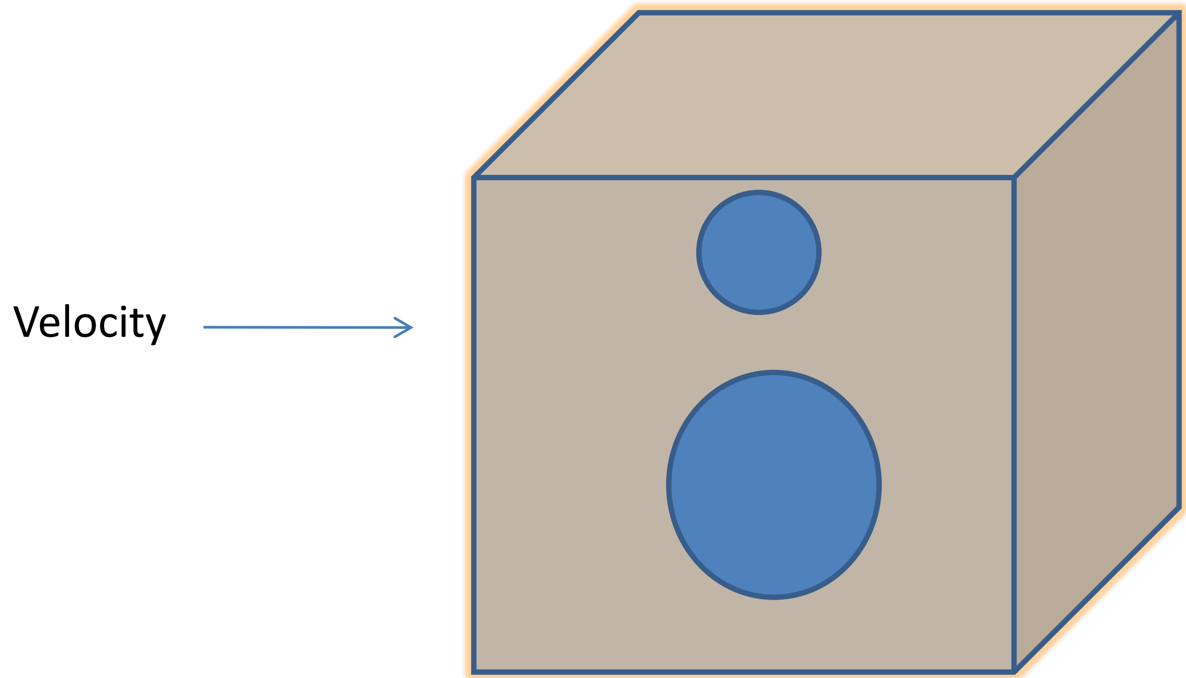
Vmax	Km	C crit	Co	D in water	D in sphere
$0.034\ mM.s^{-1}$	1.10^{-3} mM (0.7 mmHg)	1.10^{-4} mM (0.07 mmHg)	0.2 mM	$3.10^{-9}\ m^2.s^{-1}$	$2.10^{-9}\ m^2.s^{-1}$
Medium height	**δ (Heaviside)**	**Cell density**			
1 mm	flc1hs(c-0.02,0.01)	$8.8.10^{14}$ cells·m^{-3}			

$$R_{vol} = \frac{V_{\max} c}{K_m + c} . \delta$$



Simple problem solved with mass transfer equations in Comsol Multiphysics

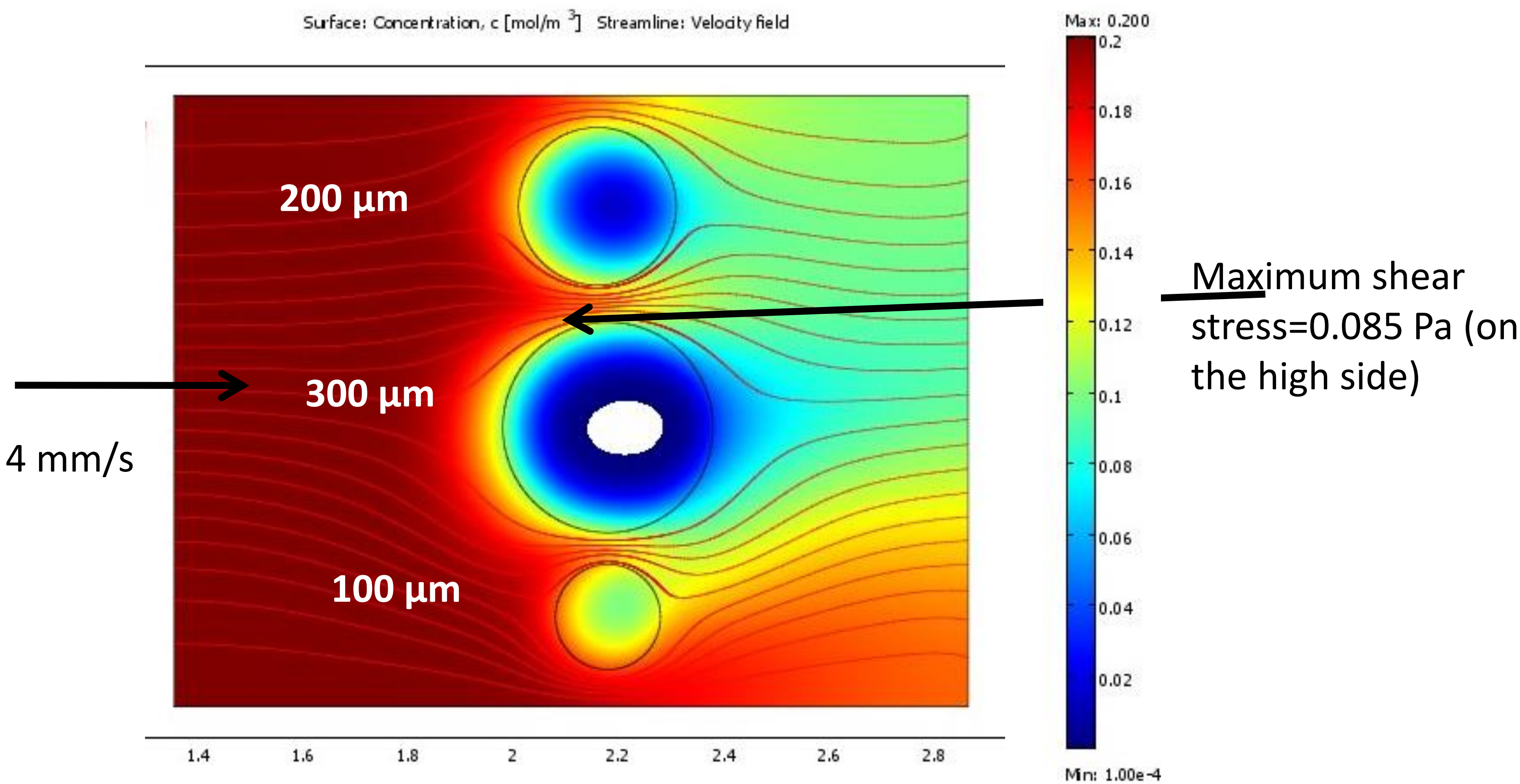
Example 2: Oxygen diffusion in perfused gel encapsulated islets in a bioreactor



The islets are encapsulated in a non porous gel
Nutrients will only get to the cells by diffusion through the gel

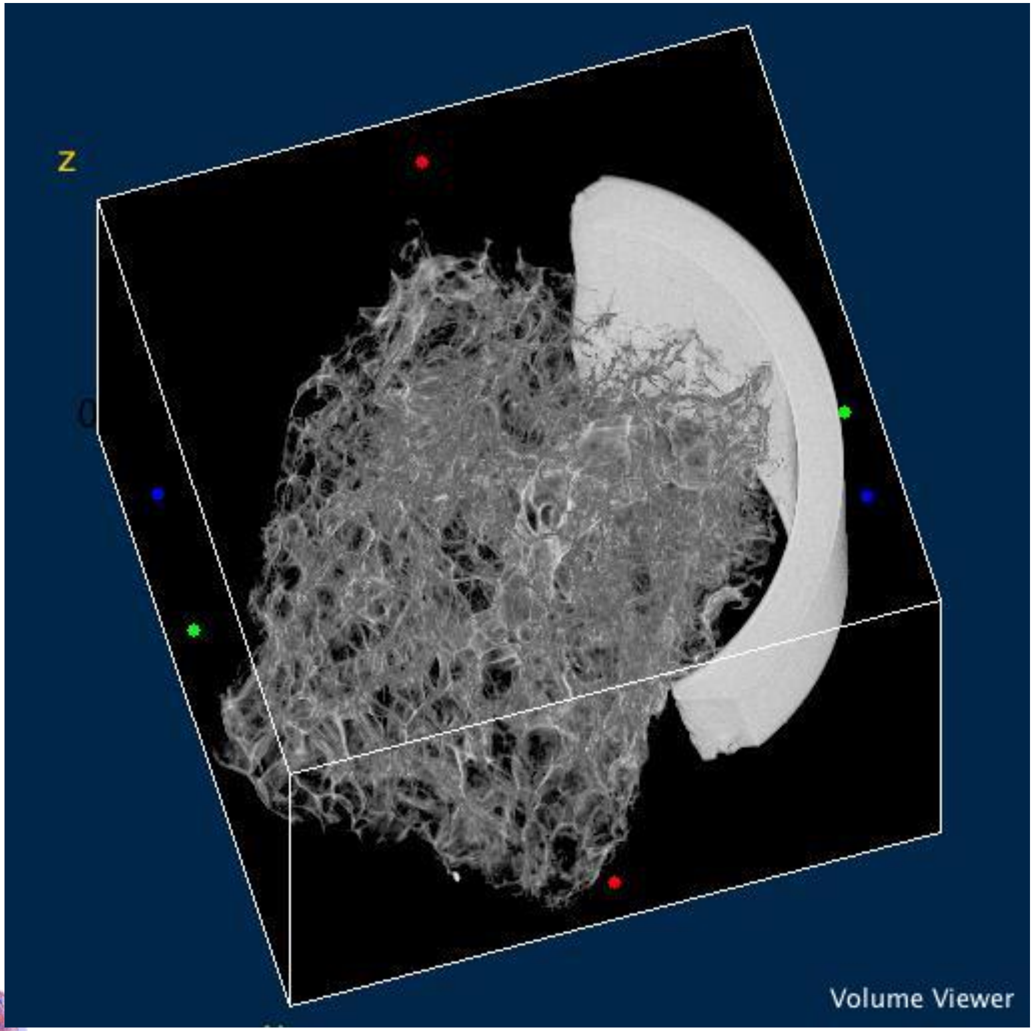
Solved by coupling the Navier-Stokes equations for the fluidic domain with convection and diffusion

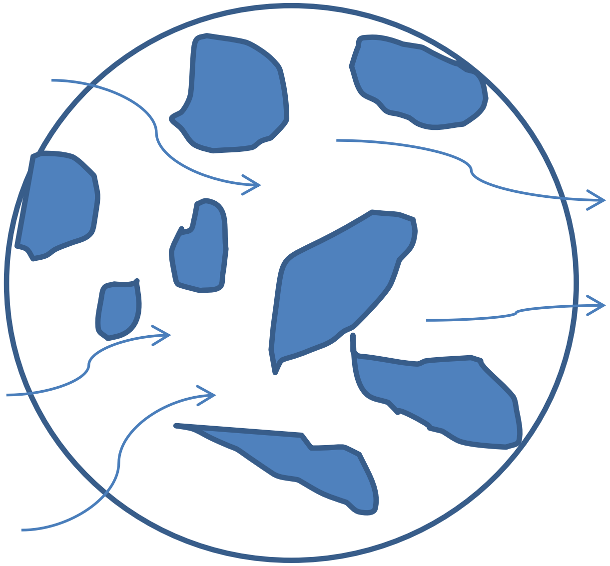
Islets in a bioreactor perfusion chamber, flow velocity of 4 mm/s.

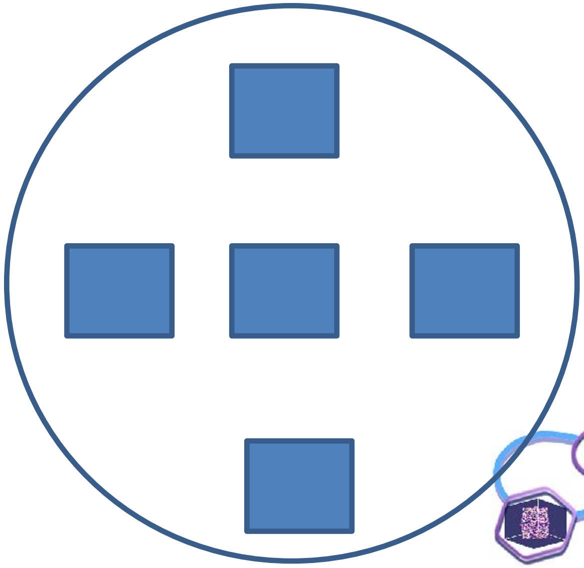


The size limit is between 200 and 300 microns
- larger constructs have to be porous

Flow through pores



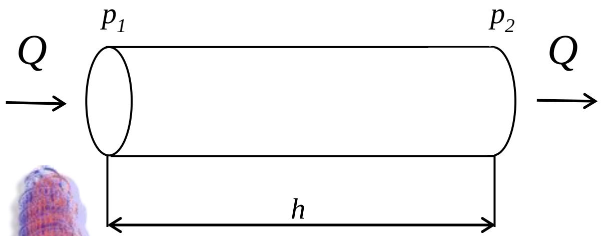


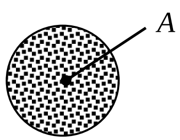


Darcy –Brinkman equations: enable calculation of average flow rate and shear in porous media, correlating pore level flow effects to the bulk fluid motion. In Darcy’s model, the average fluid velocity depends on the permeability and the pressure gradient , so the tissue is seen as a continuum with a certain resistance to flow rather than an architectured mesh.

$$\bar{\tau} = \frac{\mu Q}{A\sqrt{K_p}}$$

$$K_p = \frac{\mu Q}{A \Delta p} h$$





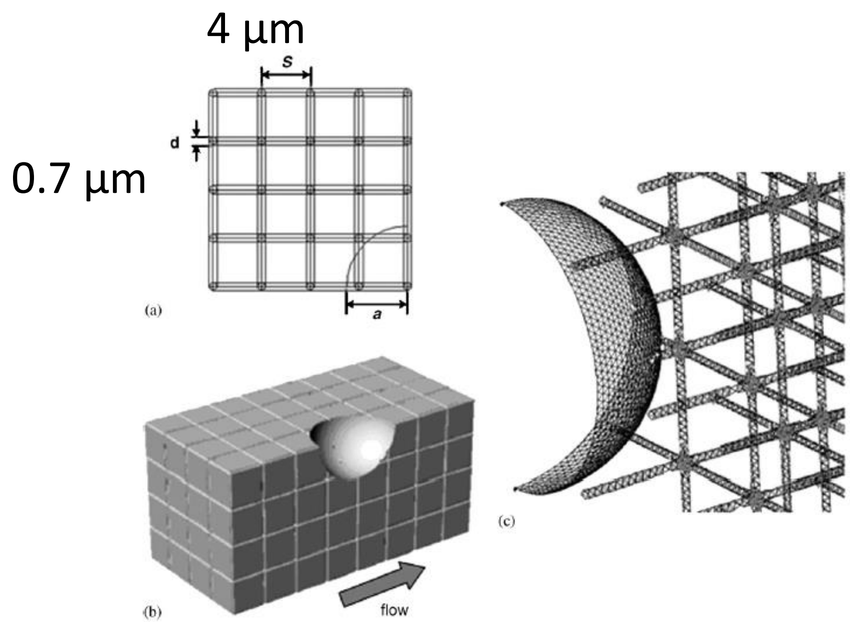
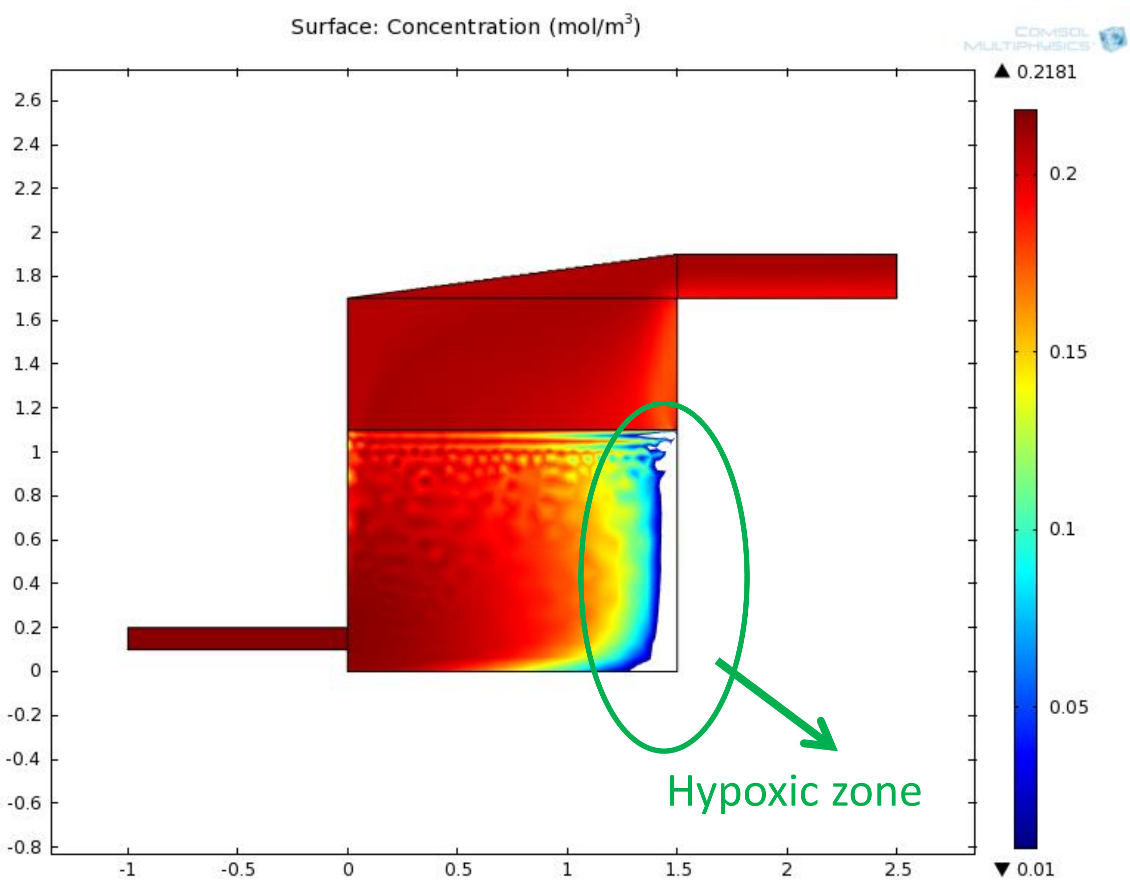


Fig. 1. CFD model setup. (a) Definition of the geometrical parameters of the model. (b) 3D rendering of the flow domain. (c) Detail of the mesh on the cell surface and nearby fibers.

Oxygen consumption

Simulation

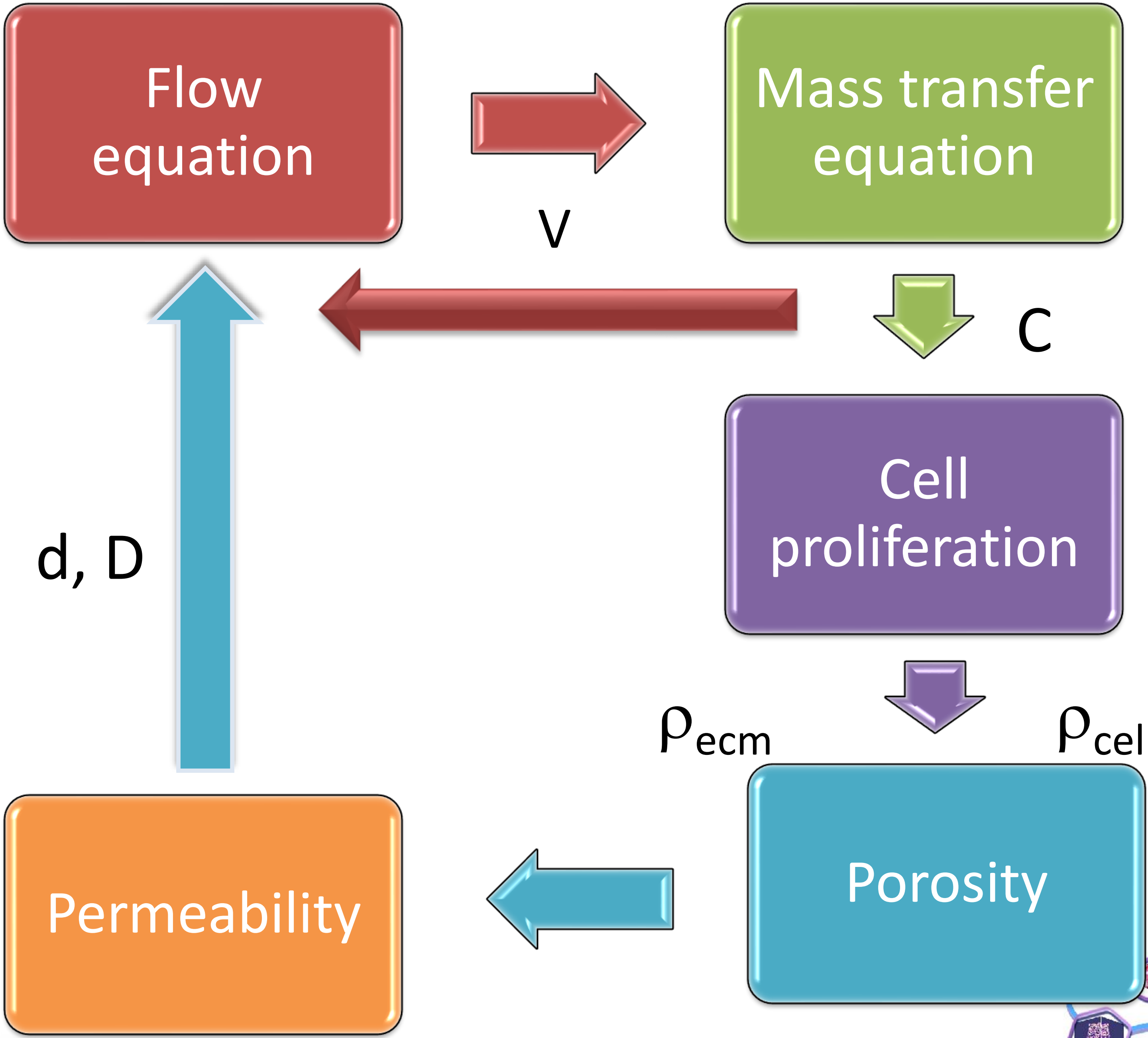
Adding reaction, and diffusion, convection multiphysics.
Sponge seeded with hepatocytes.



Reaction type Zero => constant consumption

Cell Density => $2.5.10^{-6}$ cells/cm^3

Hypoxic limit for hepatociye => 0.01 mol/m^3


Flow equation
Mass transfer equation
V
C
Cell proliferation
ρ_{ecm}
ρ_{cell}
Porosity
Permeability
d, D