“2d, 3d, shear"

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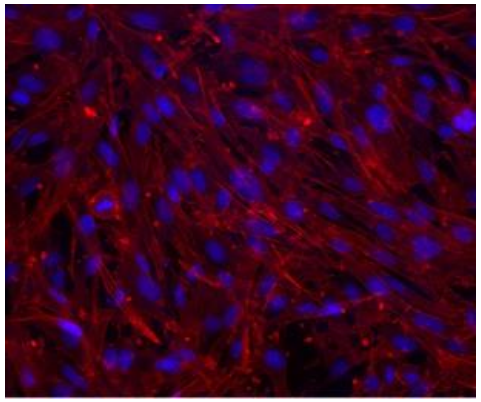
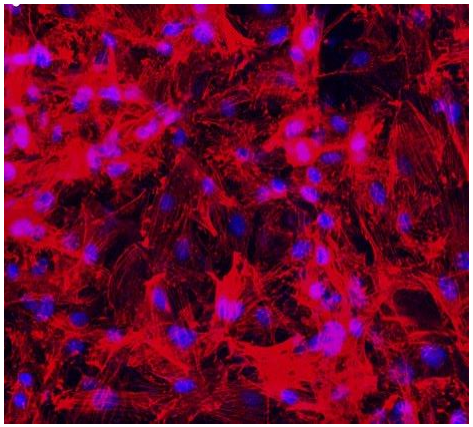
Cells don’t like 2D

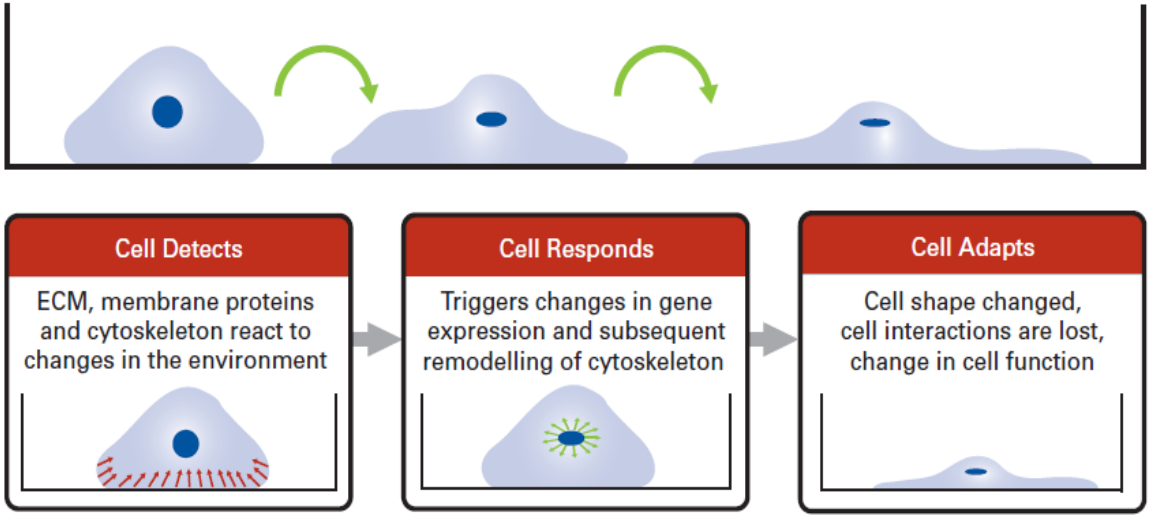


Elongated and flat in 2D



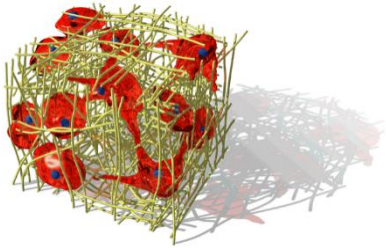
Round nuclei, cells are smaller

in vitro



3D vs 2D cultures: The evidence

	3D	2D
Shape	Ellipsoids with dimensions of 10-30 µm	Flat with typical thickness of 3 µm
Environment	~ 100 % of cell surface exposed to other cells or matrix	~ 50 % of cell surface exposed to fluid ~ 50 % exposed to the flat culture surface Very small % exposed to other cells
Behaviour	Differences in: Differentiation, Drug Metabolism, Gene and Protein expression, General Cell Function, In Vivo Relevance, Morphology, Proliferation, Response to Stimuli and Viability. 10-1000 X difference in oxygen consumption	



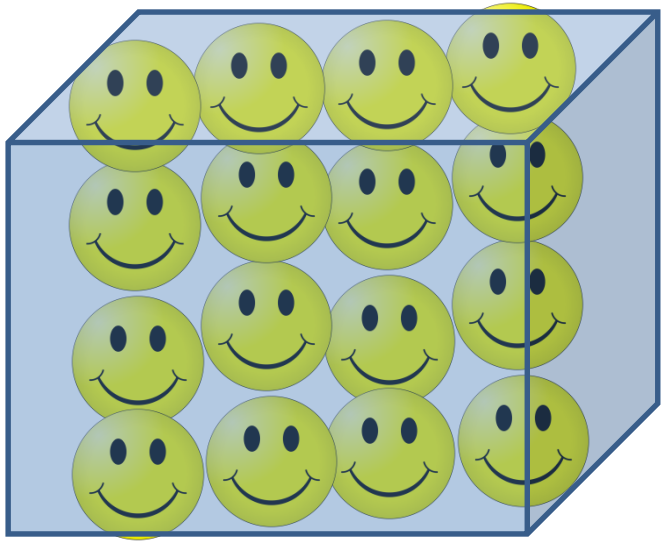
Real life happens in 3D.

So should your cell culture!

G. Mattei, S. Giusti, A. Ahluwalia. "Design criteria for generating physiologically relevant in-vitro models in bioreactors", Vol. 2, pp. 548-569, 2014, doi:10.3390/pr2030548.

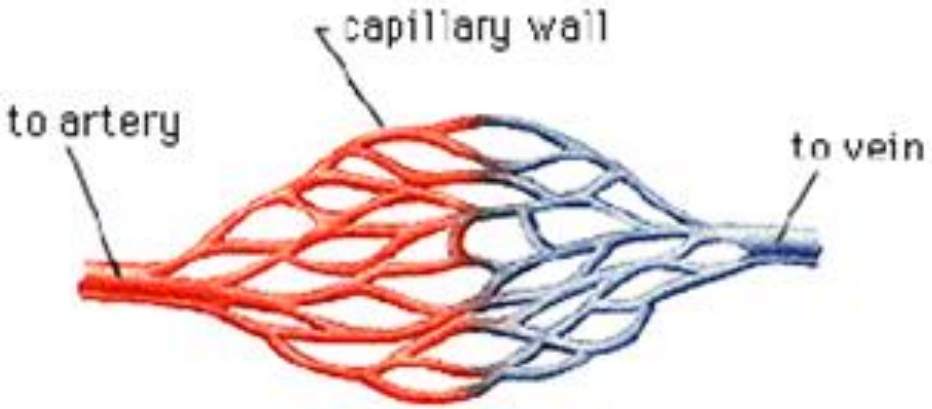
How do we define 3D?

1. Functional unit
2. 10 cell layers
3. Metabolically similar
4. Physiologically relevant

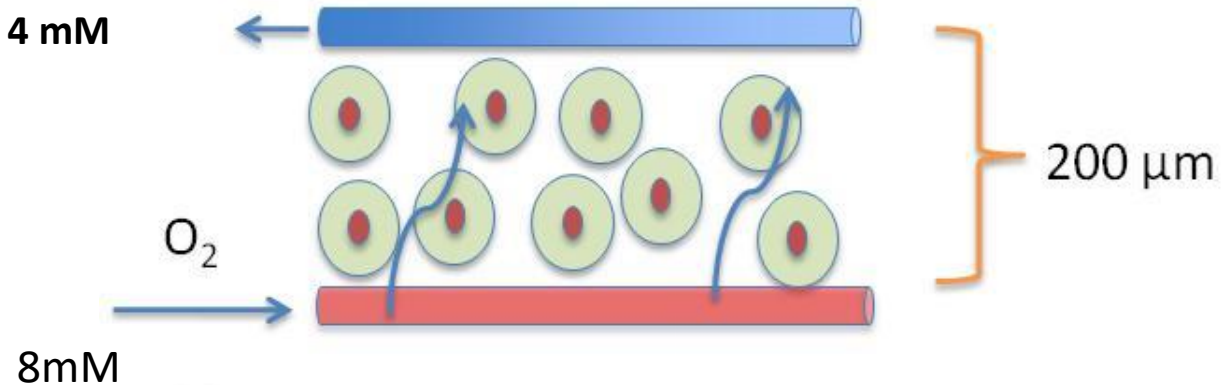


But! 3D has high oxygen demands

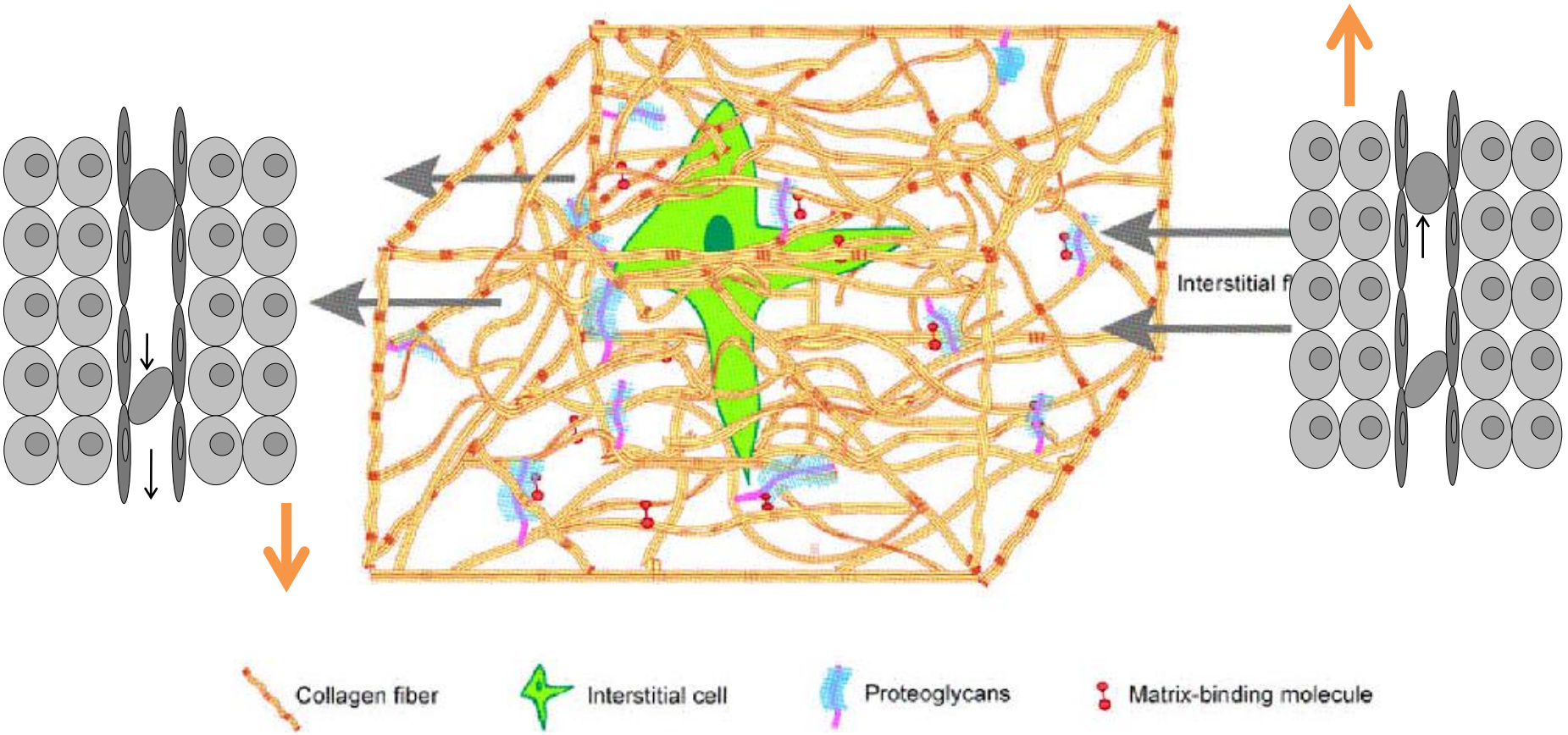
In-vivo



As O_2 diffuses through and is consumed by tissues its concentration decreases



INTERSTITIAL FLOW



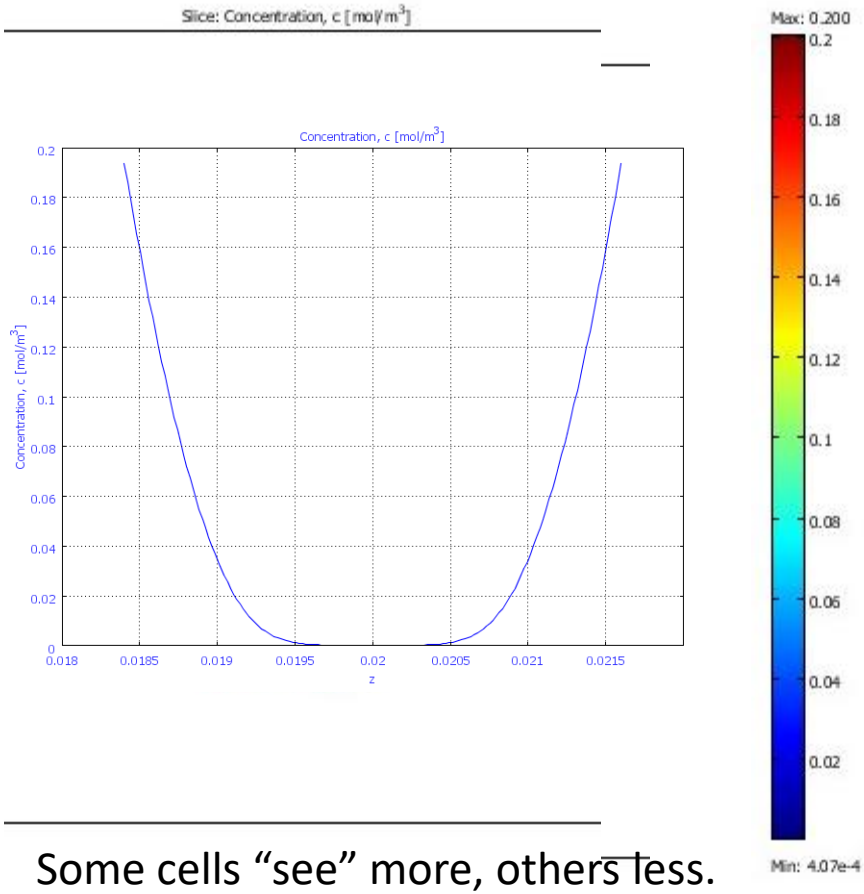
1) interstitial flow is due to a concentration gradient 2) all tissues are permeated by interstitial flow 3) the flow is through a microporous medium

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

Oxygen consumption in 2 and 3 D

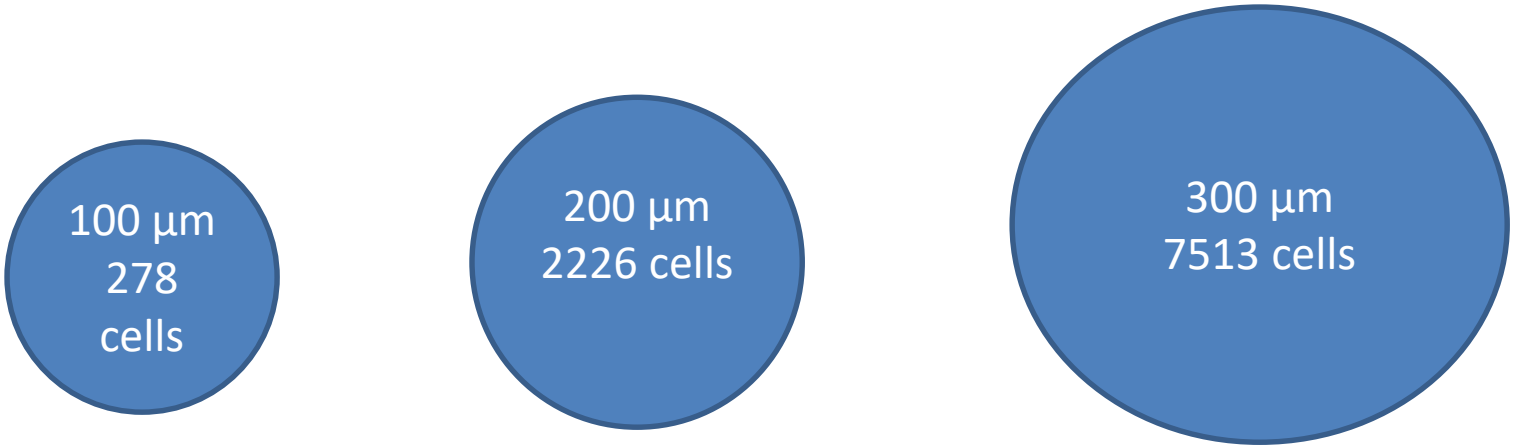
$$R_{vol} = \frac{V_{max} c}{K_m + c}$$

Consumption always zero order Vmax



Some cells “see” more, others less. Average consumption per cell is lower due to MM self regulation

Example 1: Oxygen diffusion and consumption In cell spheres, $D=3X10^{-9}$ m^2/s

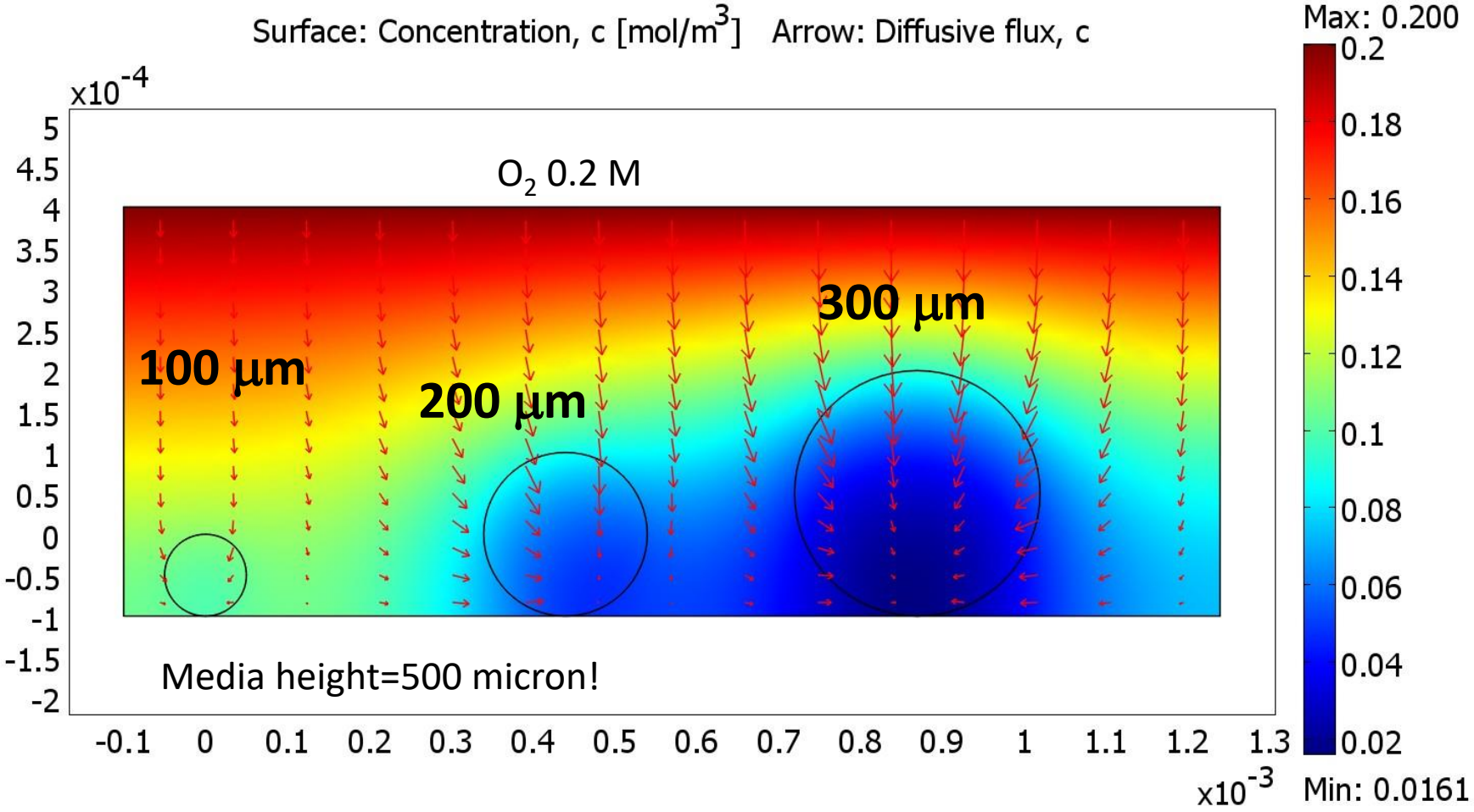


Vmax	Km	C crit	Co	D in water	D in sphere
0.034 mM.s^{-1}	**7.39.10^{-3} mM**	**1.10^{-4} mM**	**0.2 mM**	**3.10^{-9} m^2.s^{-1}**	**3.10^{-9} m^2.s^{-1}**
Medium height	**δ (Heaviside)**	**Cell density**			
1 mm	**flc1hs**	**5.3.10^{14} cells·m^{-3}**			

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

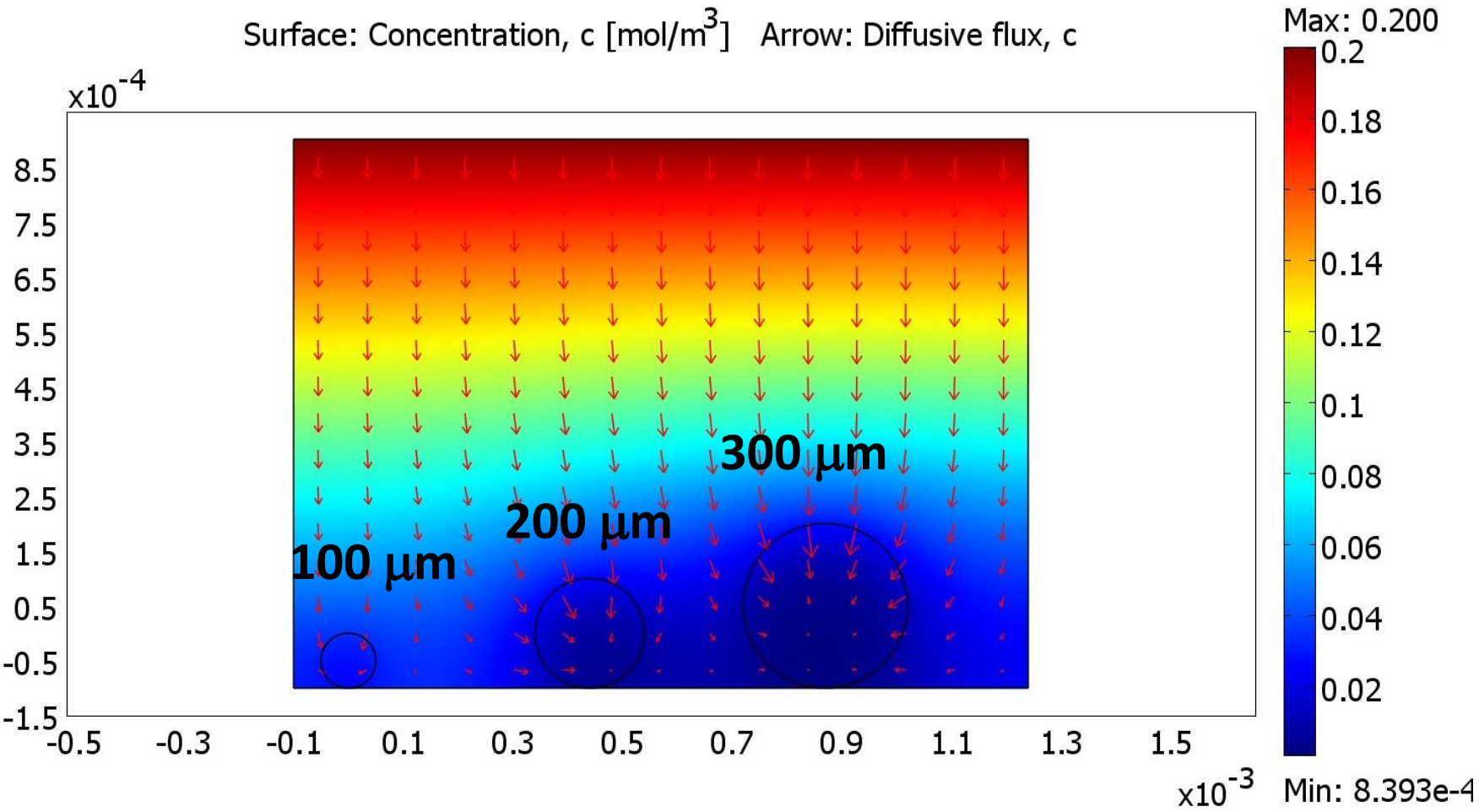
Michaelis Menten equation for oxygen consumption

Oxygen profile in 3D



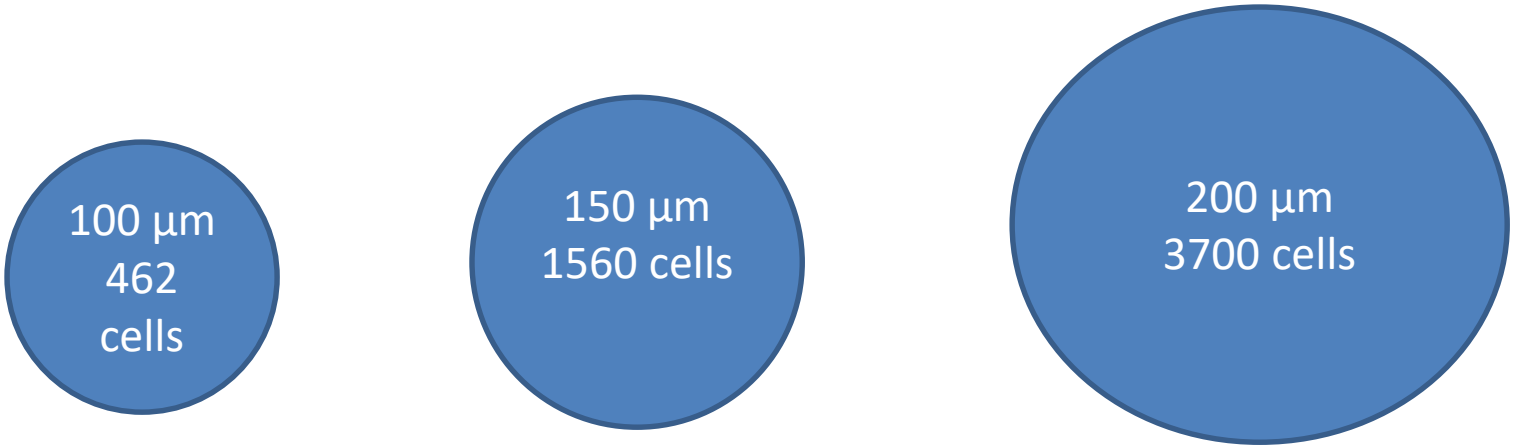
Simple problem solved with mass transfer equations in Comsol Multiphysics

Media height=1 mm



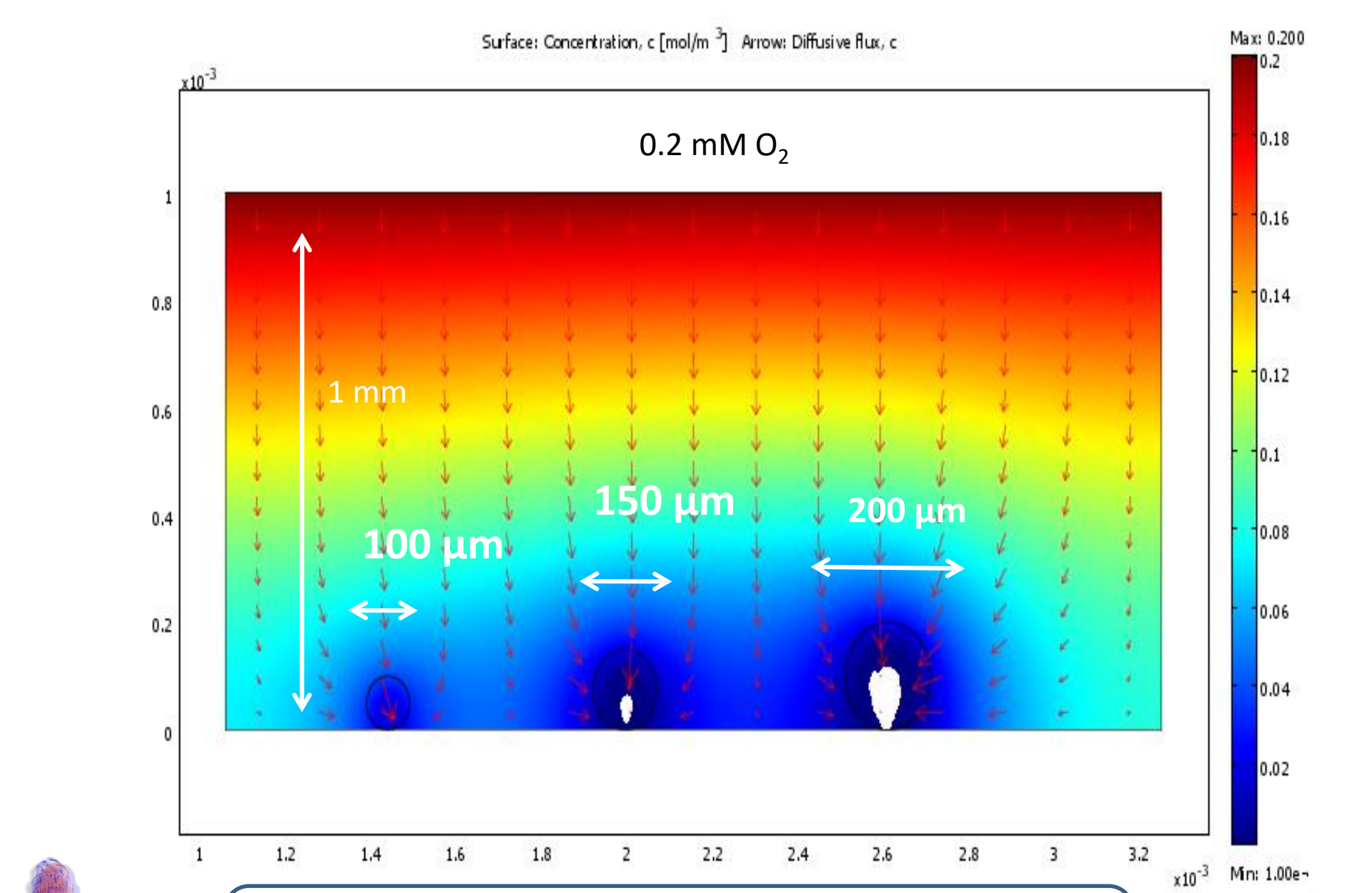
Typical heights are 5 mm, otherwise the media dries up and other nutrients may deplete

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-0.0001,0.0001/2)	5.4.10^{14} cells·m^{-3}			

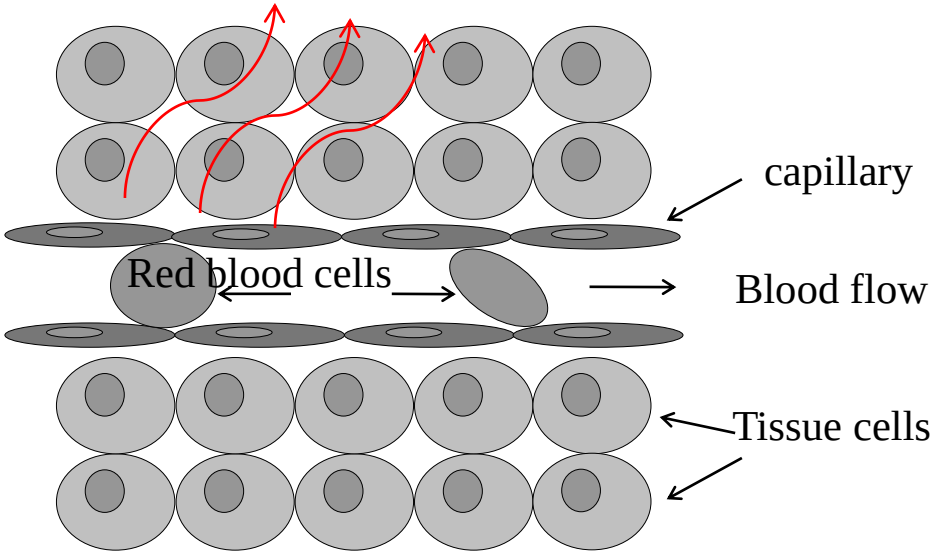
$$R_{vol} = \frac{V_{max} c}{K_m + c} . \delta$$



Simple problem solved with mass transfer equations in Comsol Multiphysics

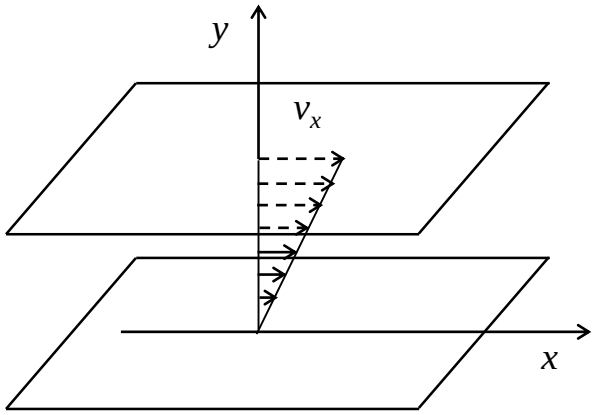
FLOW and SHEAR

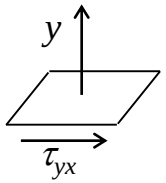
Only epithelial cells (skin, blood vessels, intestine) and the non adherent cells of the immune system and blood can support direct fluid flow.



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

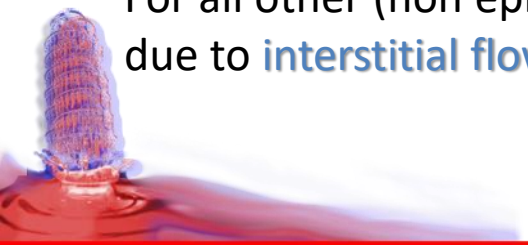
$$\tau_{yx} = -\frac{6Q\mu}{wh^2}$$

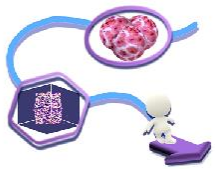
Optimal shear stress in bioreactors

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

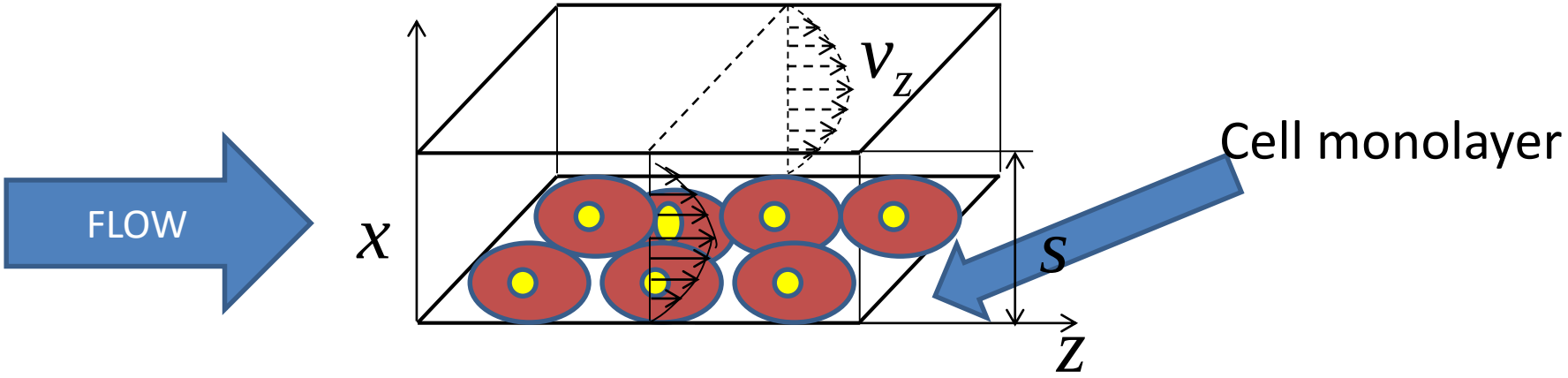
For all other (non epithelial) tissues shear is much less (0.01-0.00001 N/m^2), and is due to interstitial flow (few microL/min).





Adding flow

$$\frac{dc}{dt} = D\nabla^2 c - R_{vol} - v \cdot \nabla c$$



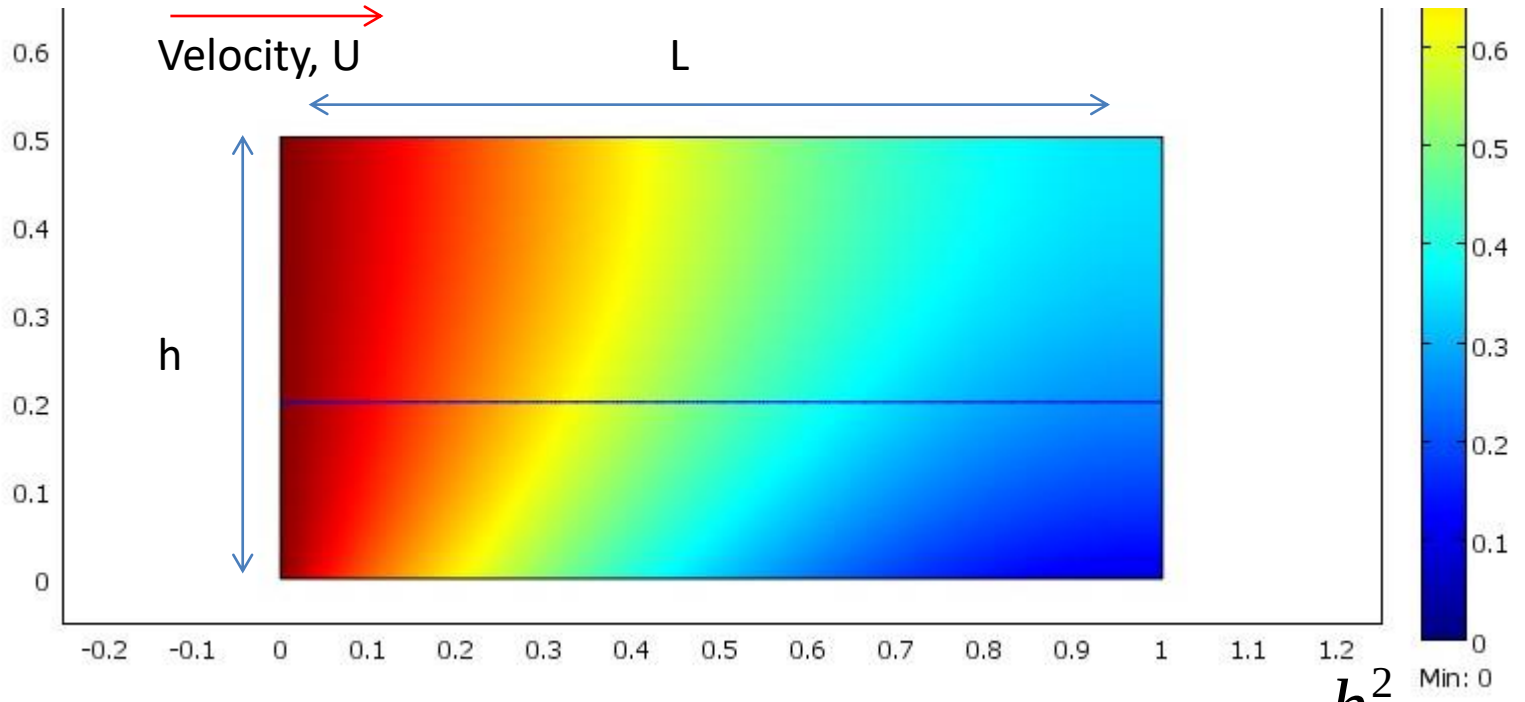
For a monolayer

$$\frac{dc}{dt} = D\frac{d^2c}{dz^2} - v_z\frac{dc}{dz}$$

For volumetric consumption

$$\frac{dc}{dt} = D\frac{d^2c}{dz^2} - v_z\frac{dc}{dz} - R_{vol}$$

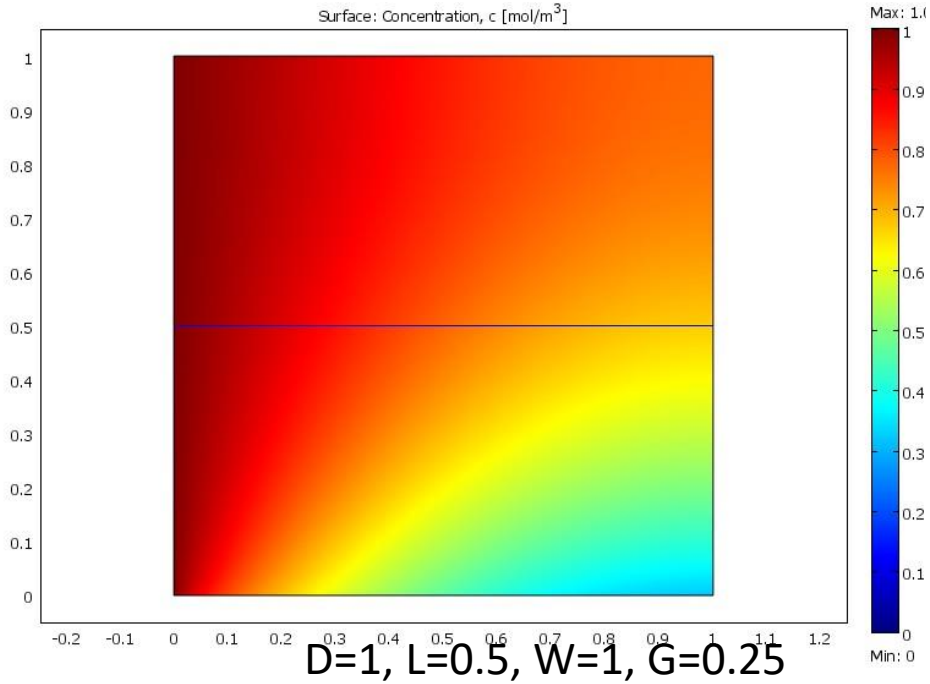
Graez number



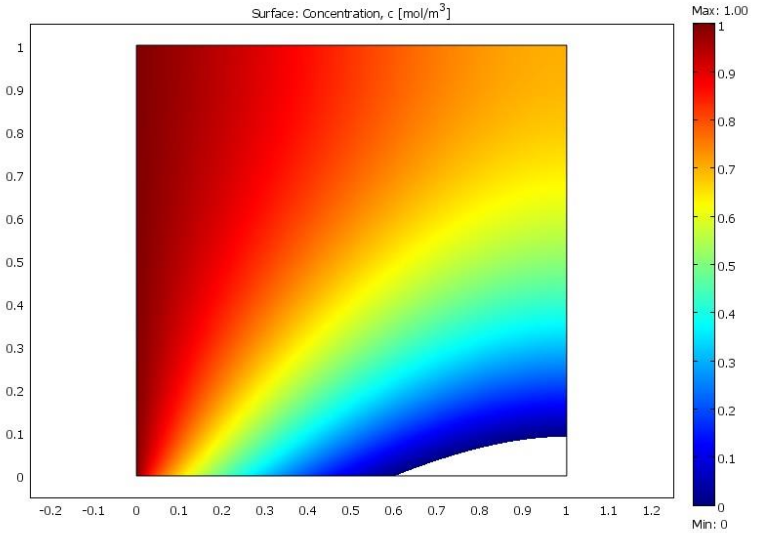
$$G = \frac{t_{diff}}{t_{conv}} = \frac{\frac{h^2}{D}}{\frac{L}{U}} = \frac{h^2 U}{DL}$$

HOW CONCENTRATION PROFILES CHANGE WITH GRAEZ NUMBER

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



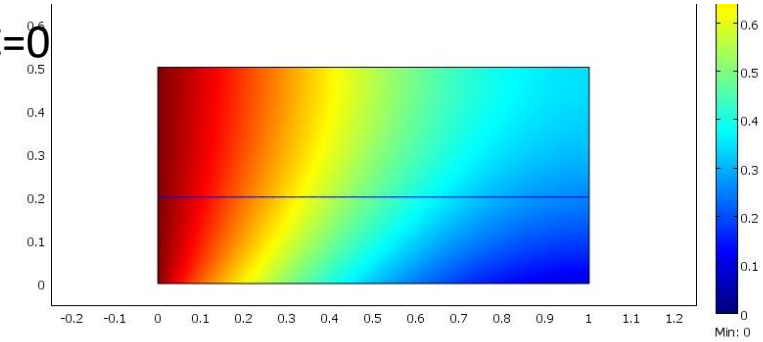
D=2, L=1, W=1, G=0.5



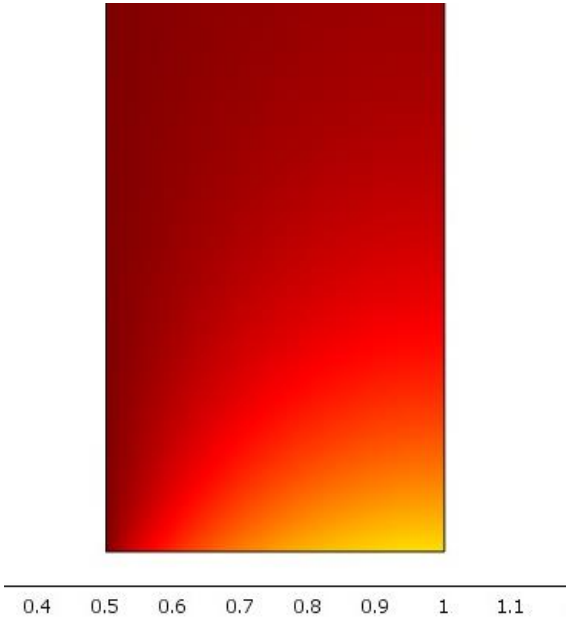
D=1, L=0.5, W=1, G=0.25

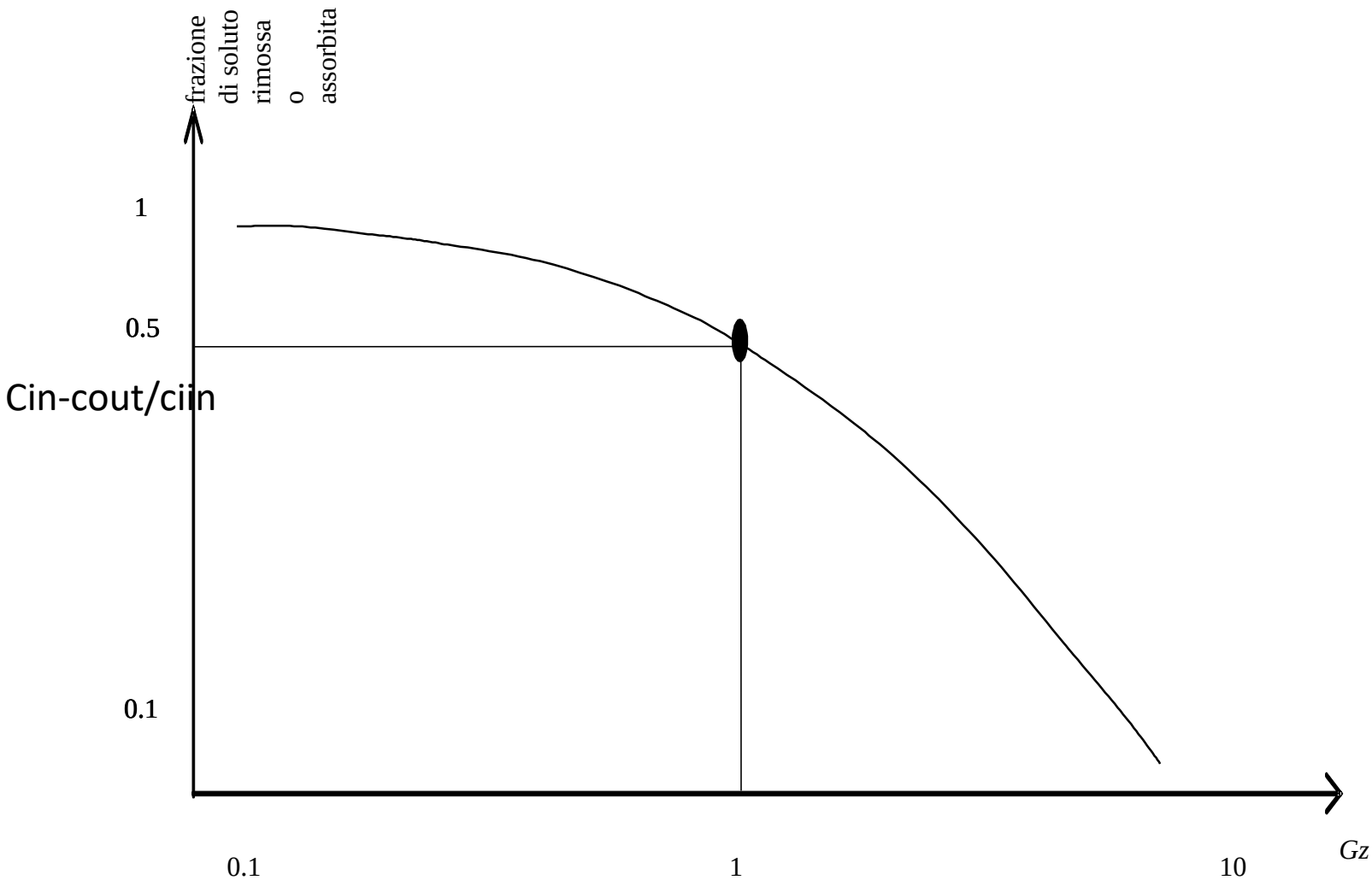
RED, C=1

BLUE C=0

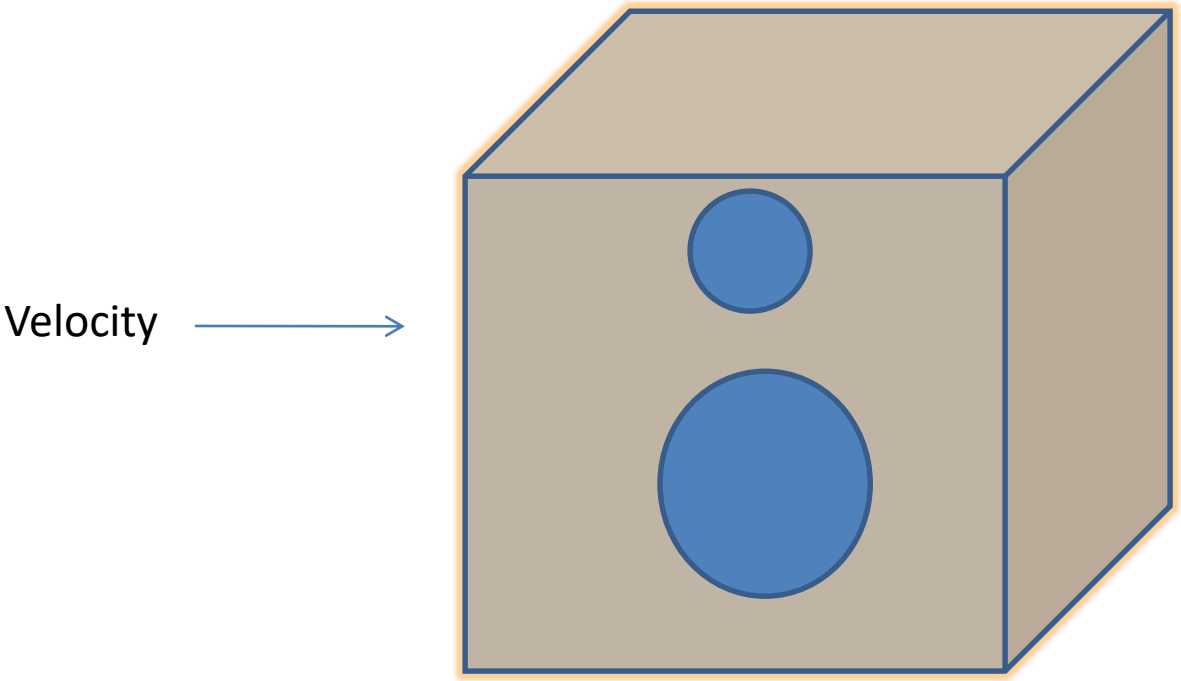


D=1, L=1, W=0.5, G=2




frazione di soluto rimossa o assorbita
1
0.5
Cin-cout/ciin
0.1
0.1
1
10
Gz

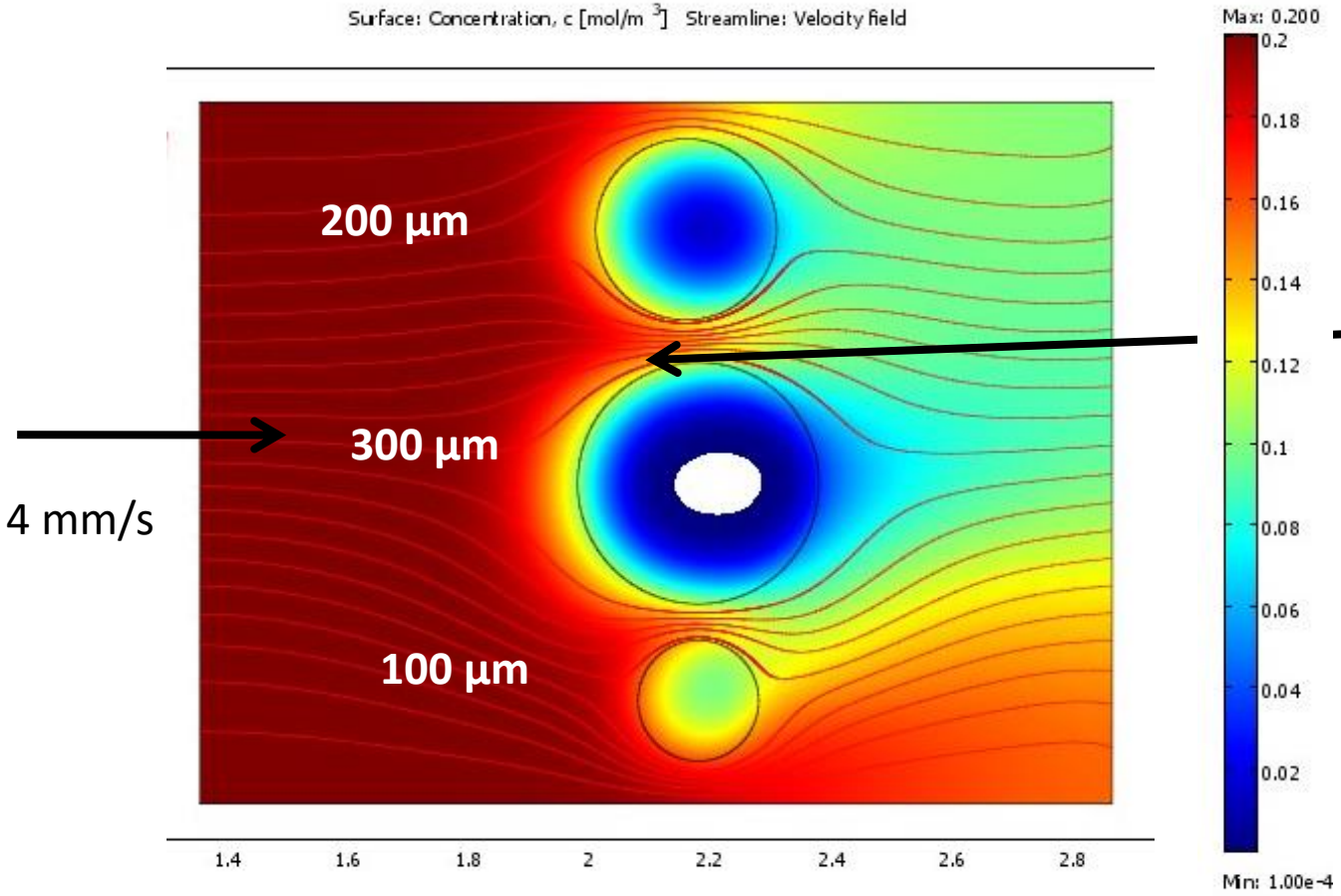
Example 2: Oxygen diffusion in perfused gel encapsulated islets in a bioreactor



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

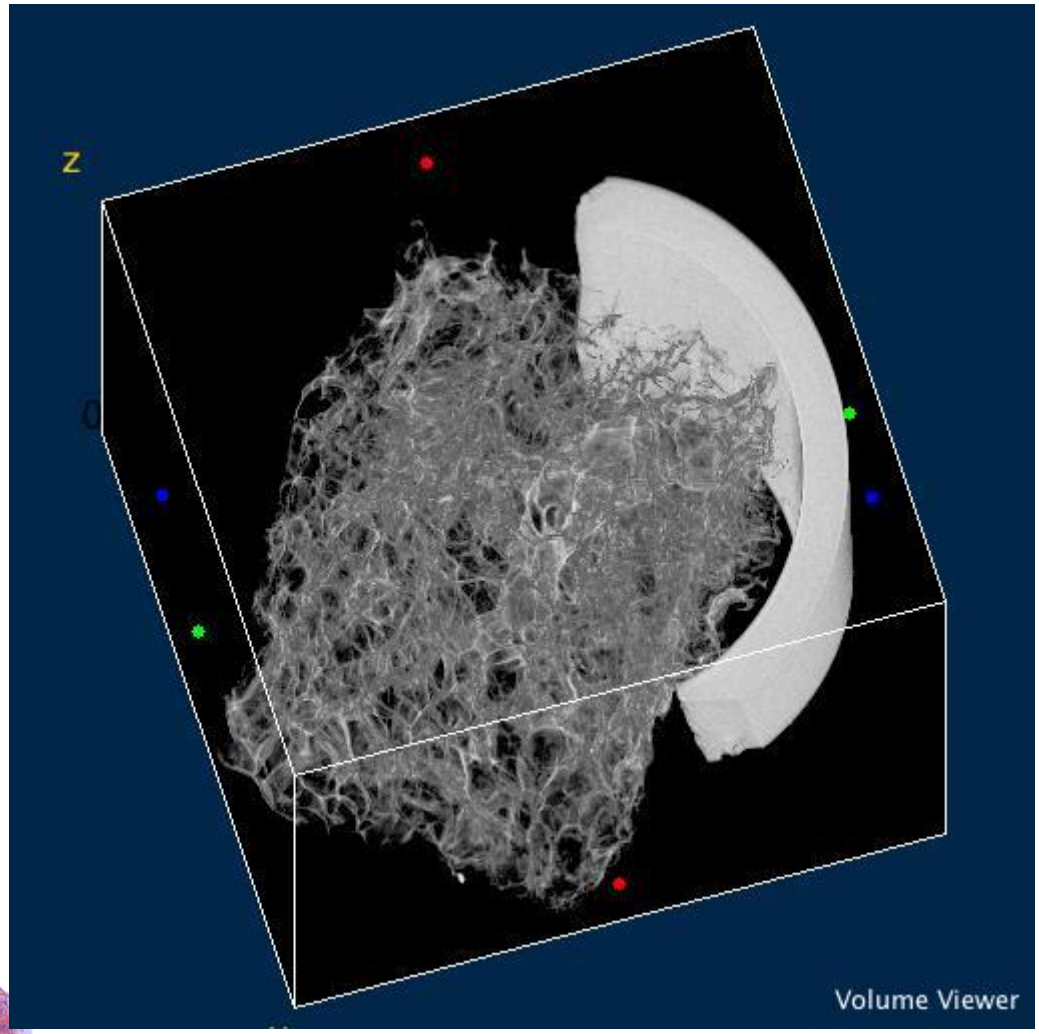
Islets in a bioreactor perfusion chamber, flow velocity of 4 mm/s.

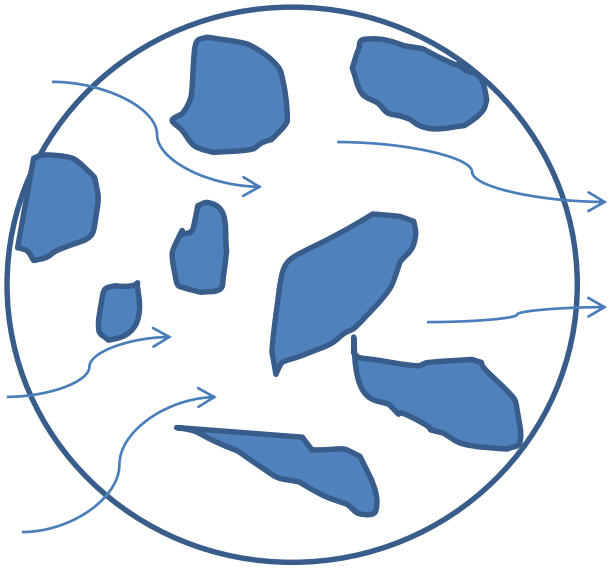


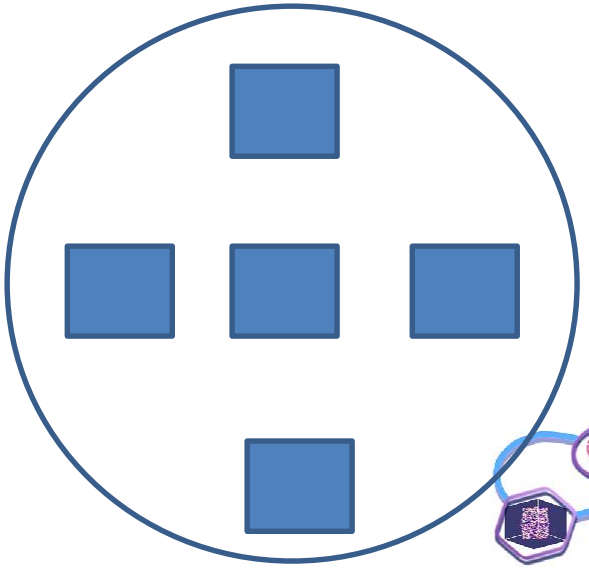
Maximum shear stress=0.085 Pa (on the high side)

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

Flow through pores



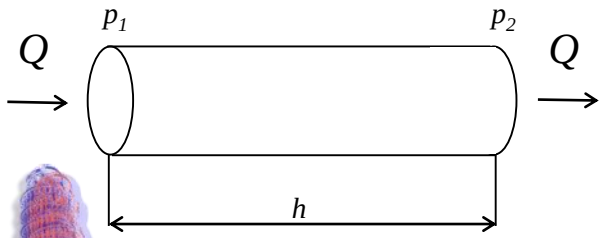


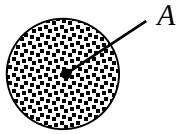


Darcy –Brinkman equations: enable calculation of average flow rate and shear in porous media, correlating pore level flow effects to the bulk fluid motion. In Darcy's model, the average fluid velocity depends on the permeability and the pressure gradient , so the tissue is seen as a continuum with a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

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

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





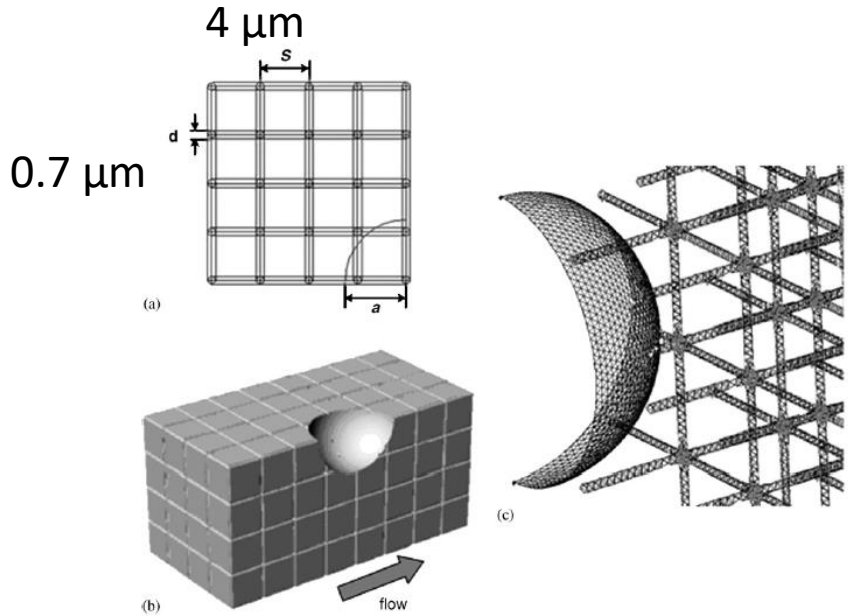
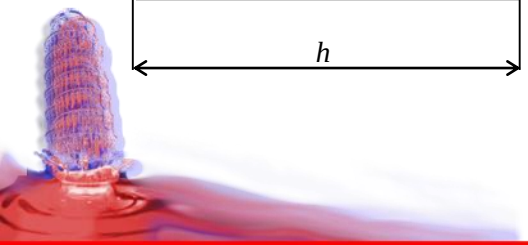
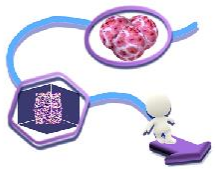


Fig. 1. CFD model setup. (a) Definition of the geometrical parameters of the model. (b) 3D rendering of the flow domain. (c) Detail of the mesh on the cell surface and nearby fibers.





The Brinkman correction to Darcys' equation takes into account the no slip condition at the walls of pores

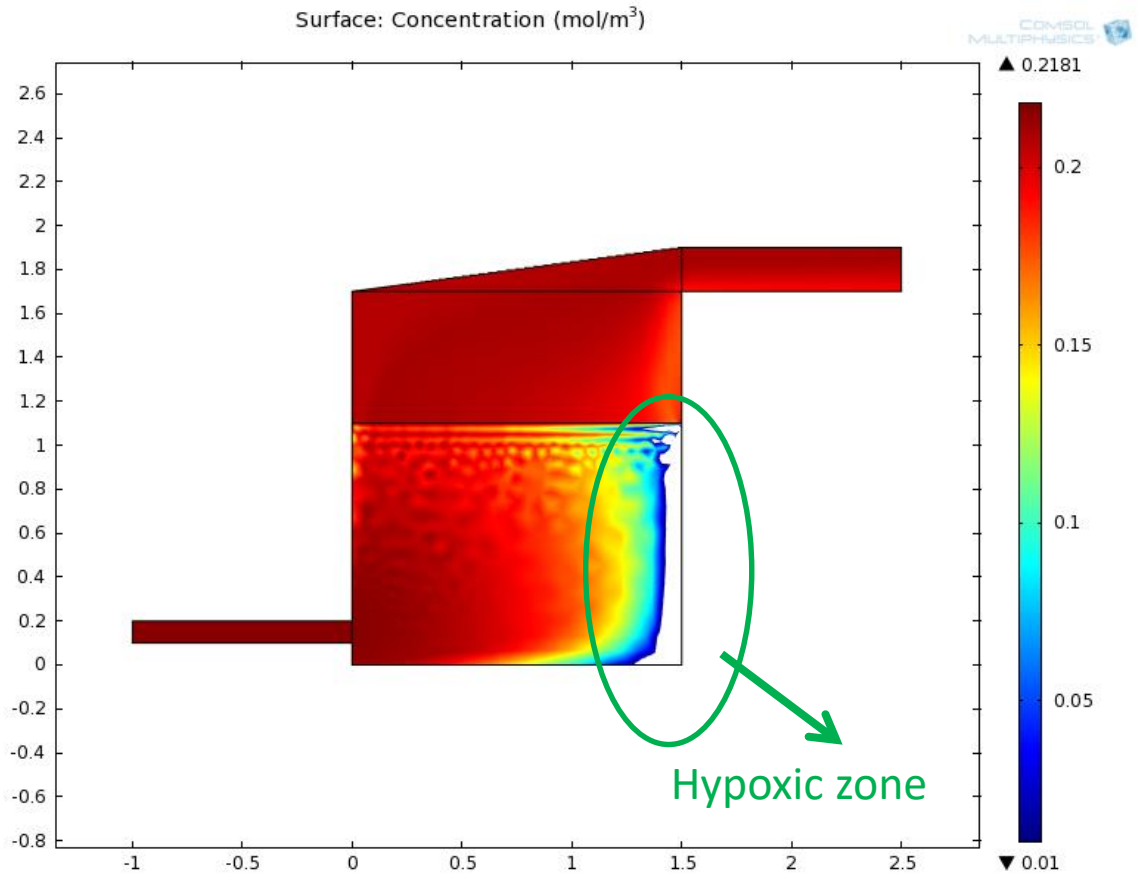
$$q = \frac{-k}{\mu} \nabla p$$

$$\mu \nabla^2 u + u = -k \nabla p$$

Oxygen consumption

Simulation

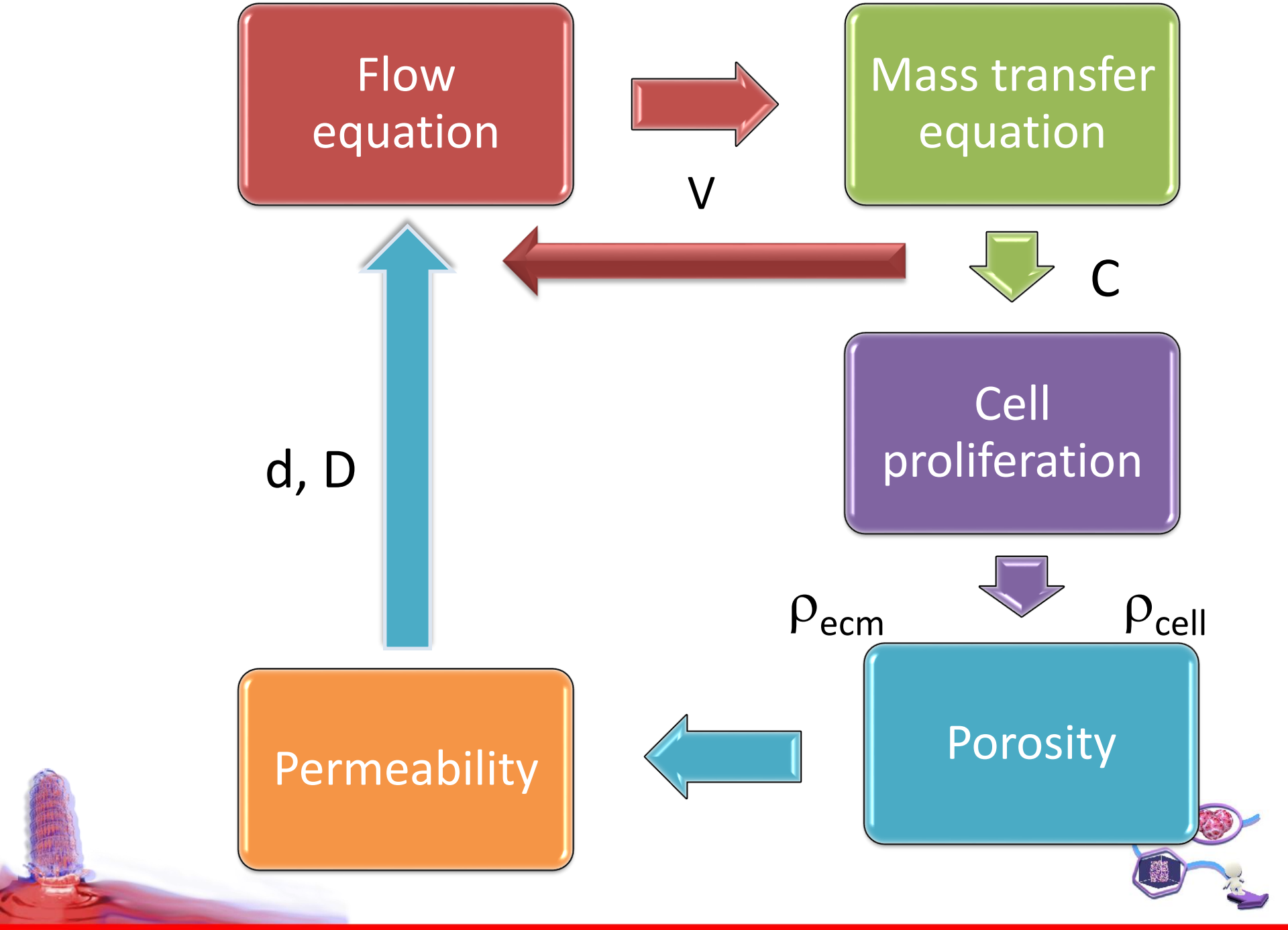
Adding reaction, and diffusion, convection multiphysics.
Sponge seeded with hepatocytes.



Reaction type Zero => constant consumption

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

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


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

OCR	Km	C crit	Co	D in water	D in sphere
1.10^{-18} to 1.10^{-16} moles.cell^{-1}.s^{-1}	**$7.39.10^{-3}$ mM**	**1.10^{-4} mM**	**0.2 mM**	**3.10^{-9} m^2.s^{-1}**	**2.10^{-9} m^2.s^{-1}**
Medium height	**δ (Heaviside)**	**Cell density in vivo**	**Vmax**	**Flow rates**	
1 mm	**flc1hs(c-crirt,crit/2)**	**$5.4.10^{14}$ cells·m^{-3}**	**Ocr*rho**	**10 to 500 µL/min**	

Cell density in body: 3.10^9 cells/mL
Cell density in vitro: 1.10^6 cells/mL