“The stress of shear"

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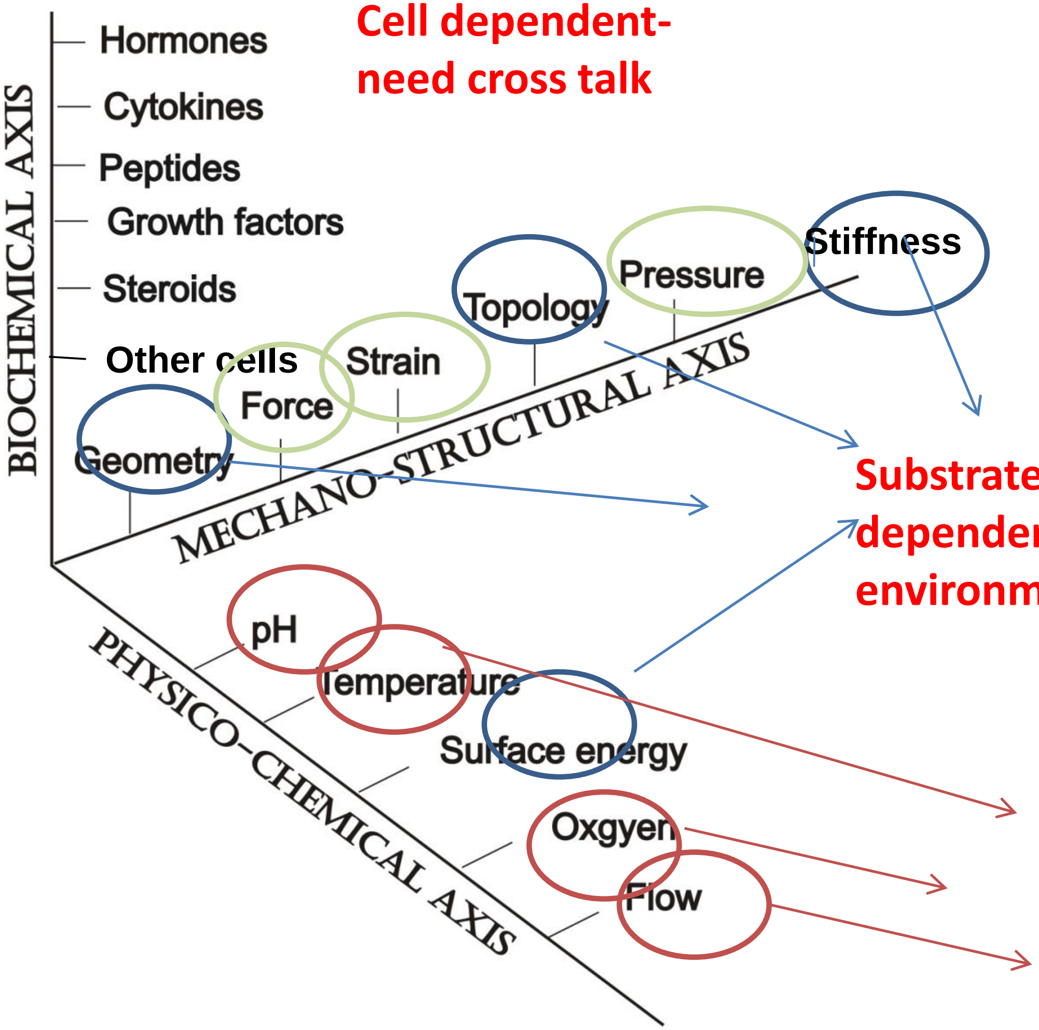
Decomposition of the cell microenvironment



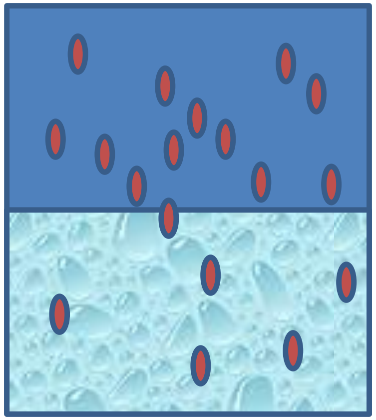
Cell dependent- need cross talk

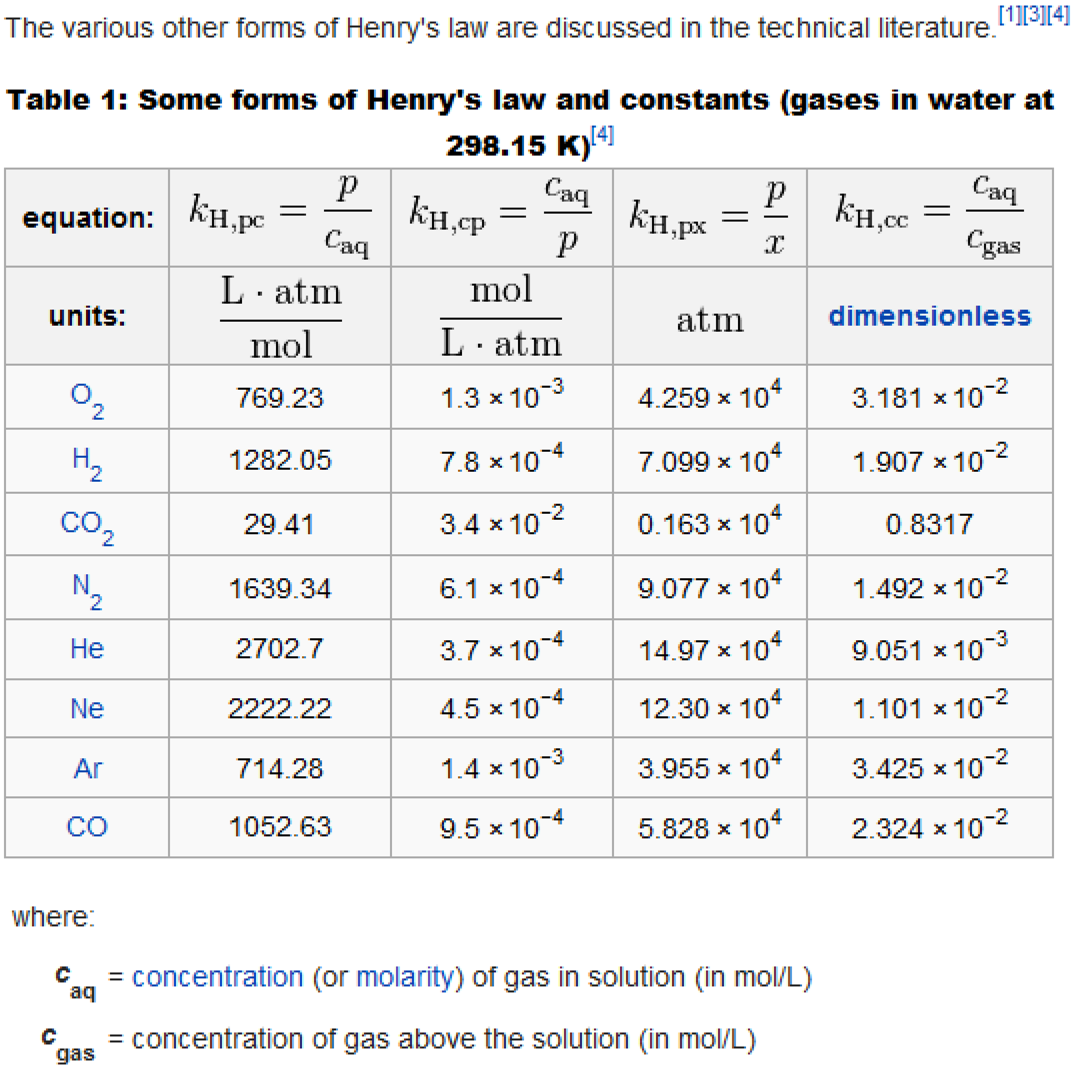
Substrate/ architecture dependent- need a 3D environment

Ambient dependent- need flow

Legge di Henry

[concentration of a gas dissolved in water]= H × [partial pressure of the gas in air]





The various other forms of Henry's law are discussed in the technical literature.[1][3][4]

Table 1: Some forms of Henry's law and constants (gases in water at 298.15 K)[4]

equation:	$k_{\mathrm{H,pc}} = \frac{p}{c_{\mathrm{aq}}}$	$k_{\mathrm{H,cp}} = \frac{c_{\mathrm{aq}}}{p}$	$k_{\mathrm{H,px}} = \frac{p}{x}$	$k_{\mathrm{H,cc}} = \frac{c_{\mathrm{aq}}}{c_{\mathrm{gas}}}$
units:	$\frac{\mathrm{L \cdot atm}}{\mathrm{mol}}$	$\frac{\mathrm{mol}}{\mathrm{L \cdot atm}}$	atm	**dimensionless**
O_2	769.23	1.3×10^{-3}	4.259×10^{4}	3.181×10^{-2}
H_2	1282.05	7.8×10^{-4}	7.099×10^{4}	1.907×10^{-2}
CO_2	29.41	3.4×10^{-2}	0.163×10^{4}	0.8317
N_2	1639.34	6.1×10^{-4}	9.077×10^{4}	1.492×10^{-2}
He	2702.7	3.7×10^{-4}	14.97×10^{4}	9.051×10^{-3}
Ne	2222.22	4.5×10^{-4}	12.30×10^{4}	1.101×10^{-2}
Ar	714.28	1.4×10^{-3}	3.955×10^{4}	3.425×10^{-2}
CO	1052.63	9.5×10^{-4}	5.828×10^{4}	2.324×10^{-2}

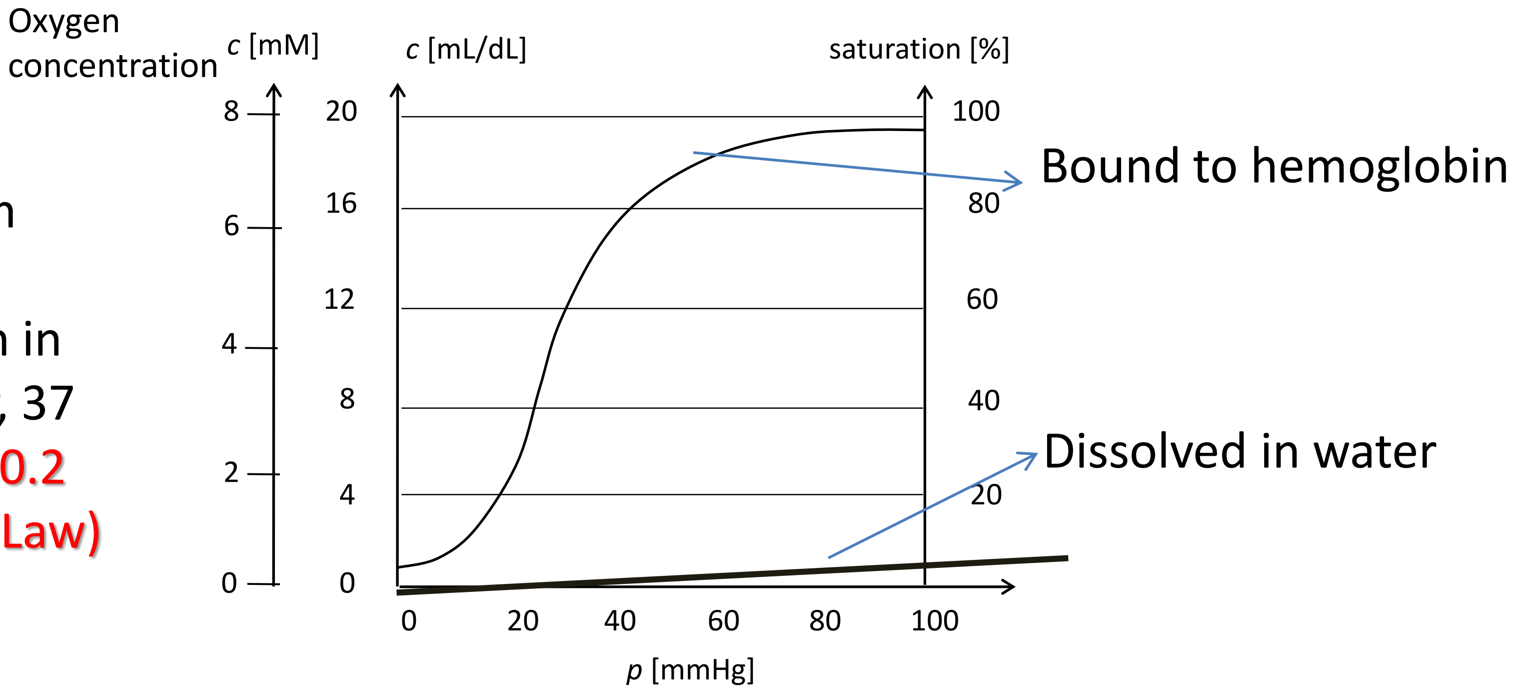
where:

c_{aq} = concentration (or molarity) of gas in solution (in mol/L)

c_{gas} = concentration of gas above the solution (in mol/L)

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)

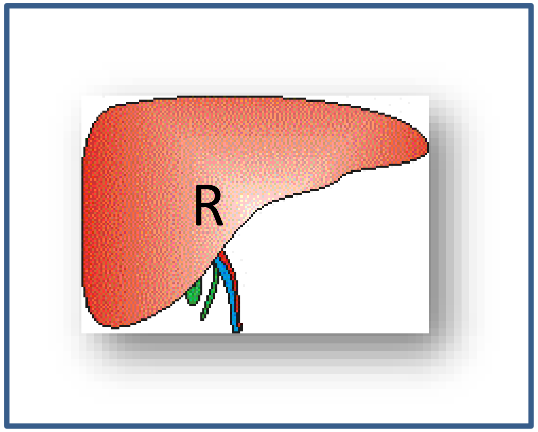


Typical concentrations

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

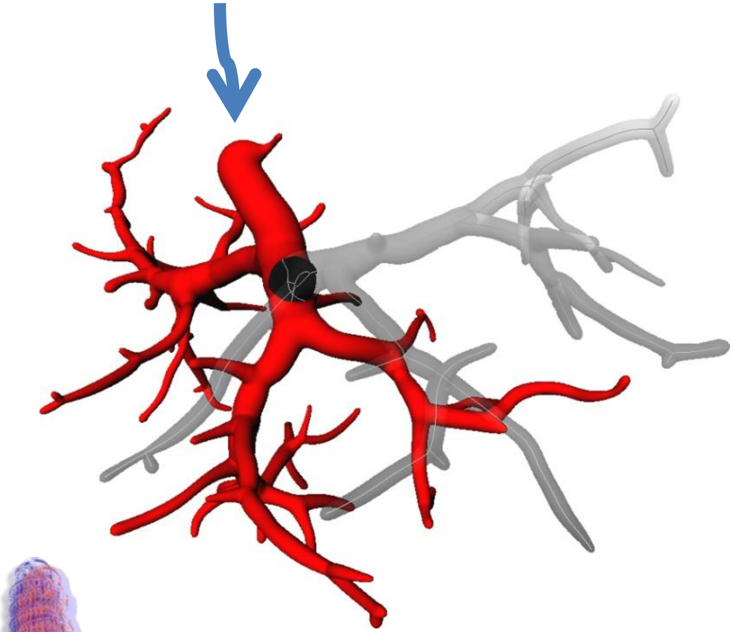
Blood flow in, Q_{in}

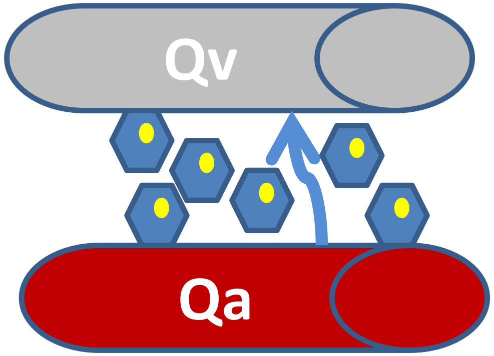
C_{in}



Blood flow out, Q_{out}

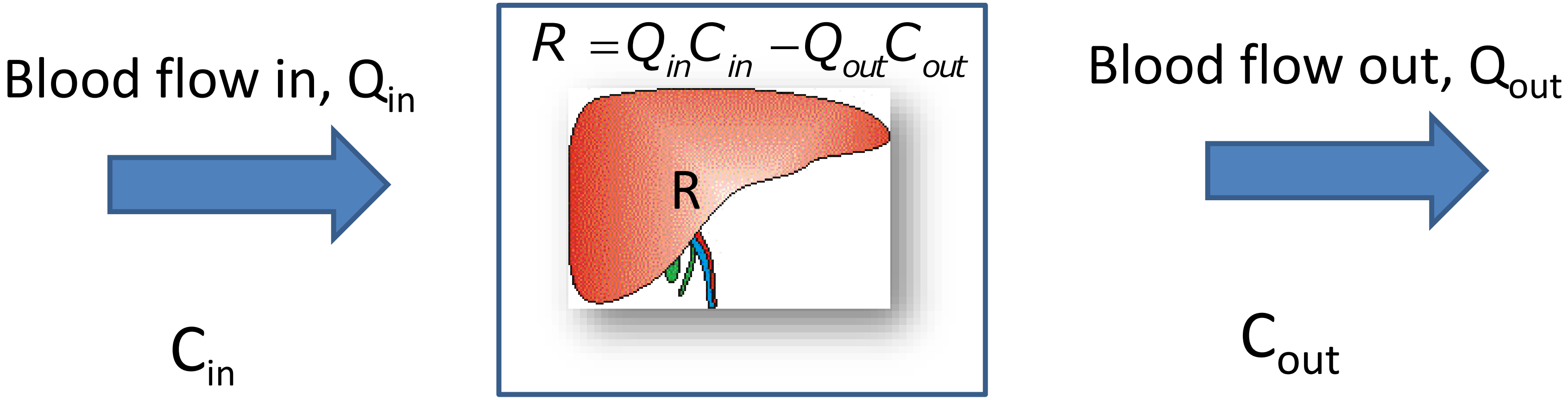
C_{out}





Interstitial flow driven by concentration gradients

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)

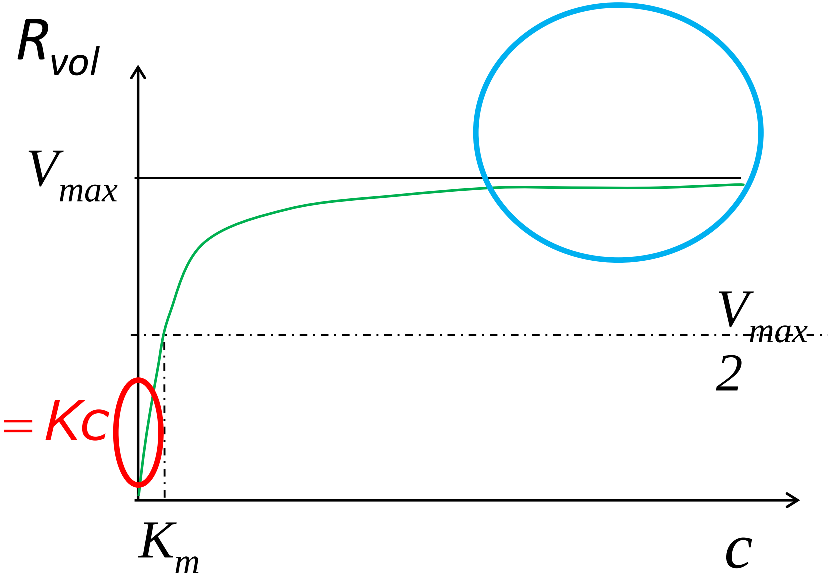
R_c=specific consumption (moles.s^{-1}/cell)

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

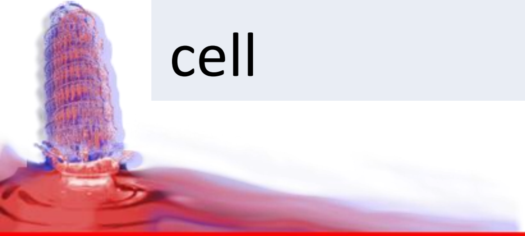
$$R_{vol} = V_{\max}$$

First order

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

Oxygen consumption rates

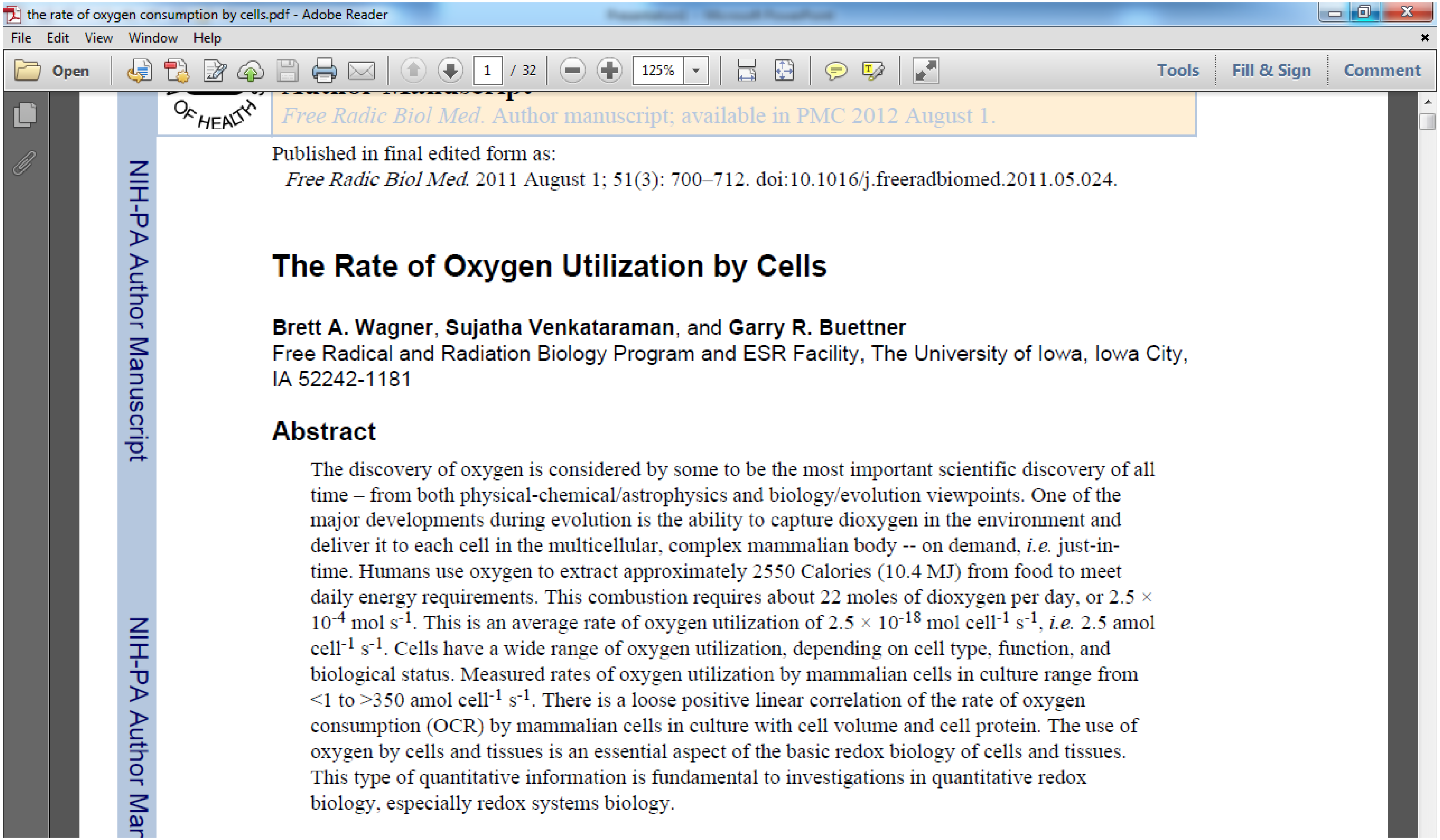
Organ/tissue	R	Rs (moles.s^{-1}/cell) or OCR
Whole body	260 mL O_2/min → (5×10^{13} cells)	3×10^{-17}
Liver	58 mL O_2/min→ (2×10^{11} hepatocytes)	3×10^{-16}
Cartilage		3×10^{-19}
Bone marrow Stem cell		1.5×10^{-17}



Data for estimating average OCR (oxygen consumption rate) per cell in the body

- 12 breaths/min
- Each breath is 500 mL
- O2 is 150 mmHg in, 40 mmHg out
- PV=nRT

Data for estimating average OCR (oxygen consumption rate) per cell in the body



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The Rate of Oxygen Utilization by Cells

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Abstract

The discovery of oxygen is considered by some to be the most important scientific discovery of all time – from both physical-chemical/astrophysics and biology/evolution viewpoints. One of the major developments during evolution is the ability to capture dioxygen in the environment and deliver it to each cell in the multicellular, complex mammalian body -- on demand, *i.e.* just-in-time. Humans use oxygen to extract approximately 2550 Calories (10.4 MJ) from food to meet daily energy requirements. This combustion requires about 22 moles of dioxygen per day, or 2.5×10^{-4} mol s^{-1}. This is an average rate of oxygen utilization of 2.5×10^{-18} mol $cell^{-1}$ s^{-1}, *i.e.* 2.5 amol $cell^{-1}$ s^{-1}. Cells have a wide range of oxygen utilization, depending on cell type, function, and biological status. Measured rates of oxygen utilization by mammalian cells in culture range from <1 to >350 amol $cell^{-1}$ s^{-1}. There is a loose positive linear correlation of the rate of oxygen consumption (OCR) by mammalian cells in culture with cell volume and cell protein. The use of oxygen by cells and tissues is an essential aspect of the basic redox biology of cells and tissues. This type of quantitative information is fundamental to investigations in quantitative redox biology, especially redox systems biology.

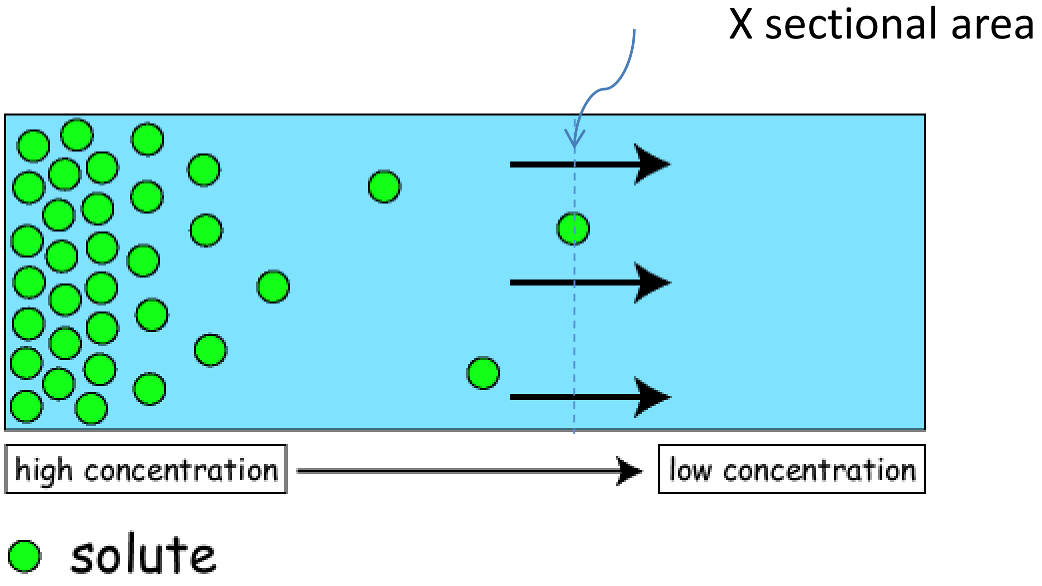
energy in light from the sun is captured so protons and electrons can be combined with CO_2 to synthesize $(CHO)_n$, (high energy bonds) providing the foundation for the carbon-chemistry of life -- photosynthesis. In Rxn **2** those carbon-based compounds are "burned" to provide the energy of life -- respiration. The enzymatic systems of cells carefully control this combustion process. As these electrons and protons are put onto dioxygen to form water, the energy of combustion is captured to do the synthesis, repair, and work needed for life.

Dioxygen is not stored in the body; rather the air (or water) of the environment is the immediate reservoir and omnipresent source of dioxygen. One of the major developments during evolution is the ability to extract oxygen from the environment and deliver it to each cell in the multicellular, complex mammalian body -- on demand, *i.e.* just-in-time.

Humans use this oxygen to extract approximately 2550 Calories (10.4 MJ for a 70 kg, 20 y old male [5]) from food to meet daily energy requirements. This combustion requires approximately 22 moles of dioxygen per day, or 2.5×10^{-4} mol s^{-1}. For a 70 kg person, this rate of O_2-uptake is 3.6×10^{-9} mol s^{-1} g^{-1}. If the typical 70 kg person consists of 1×10^{14} cells, then the average rate of oxygen utilization per cell would be 2.5×10^{-18} mol $cell^{-1}$ s^{-1}, *i.e.* 2.5 amol $cell^{-1}$ s^{-1}. Cells have a wide range of oxygen utilization, depending on cell type, function, and biological status. One would expect the oxygen utilization of a relatively large hepatocyte with on the order of 10^3 mitochondria [6] to be very different than a small red blood cell with no mitochondria, which relies totally on glycolysis rather than respiration for its energy needs.

The vast majority of the dioxygen used in mitochondrial respiration undergoes four-electron reduction to produce water, Rxn 2. A small fraction undergoes one-electron reduction to form superoxide, estimated to ≈1 %, or less of the OCR [7, 8, 9, 10]; the actual univalent reduction of dioxygen in the electron transport chain of the mitochondrion *in vivo* is thought to be much less than this [7]. This superoxide is thought to be primarily produced by the reaction of dioxygen with the semiquinone radical ($CoQ^{\bullet -}$) of coenzyme Q (ubiquinone) of

Diffusion

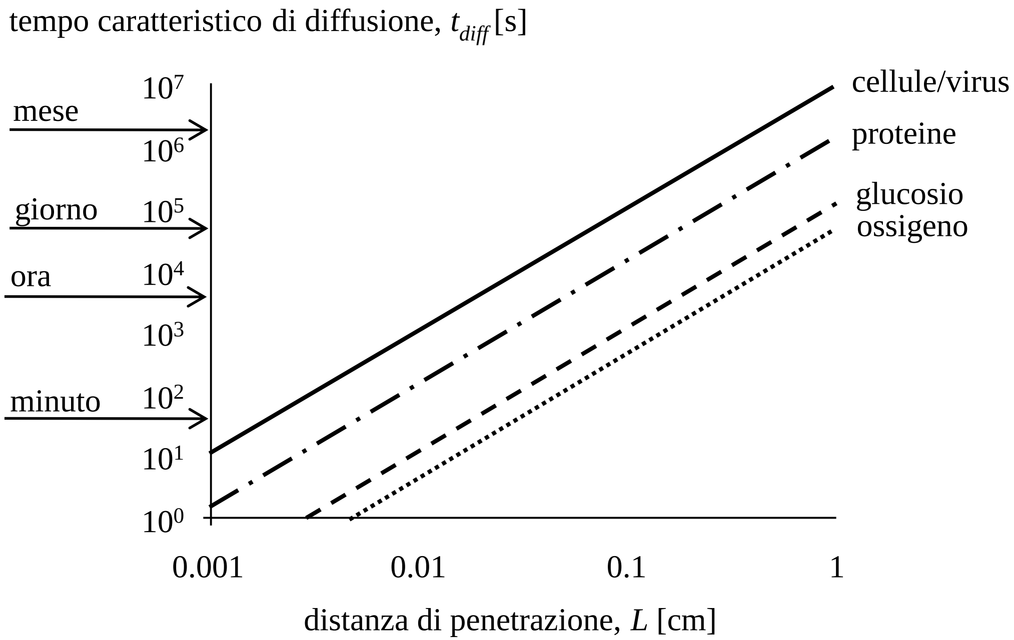


Solute transport is due to the concentration gradient dc/dx.

J is molecular flux rate across unit surface area (moles/m^2/s).

D is the diffusion constant (m^2/s)

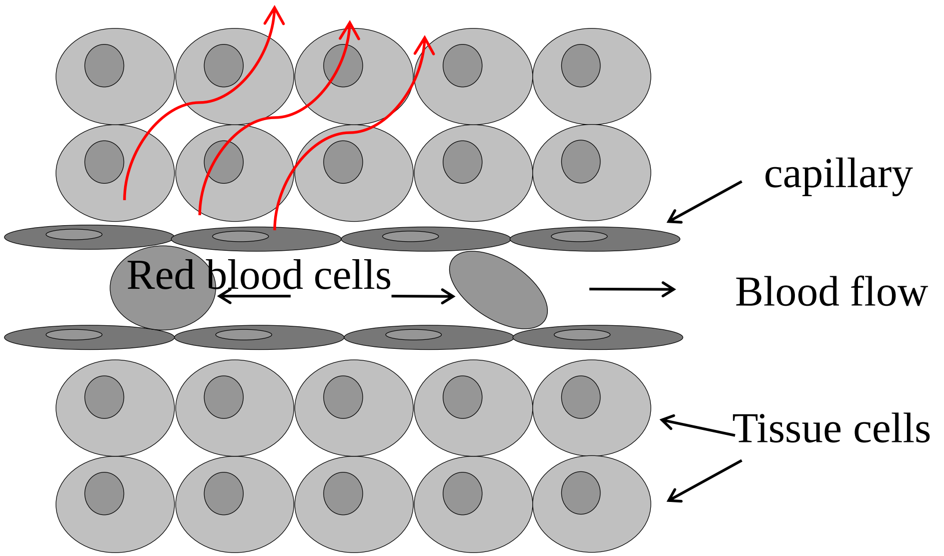
$$J = -D\frac{dc}{dx}, \qquad \frac{dc}{dt} = D\frac{d^2c}{dx^2}$$



$$t_{diff} = \frac{L^2}{D}, D = \frac{KT}{6\pi\mu r}$$

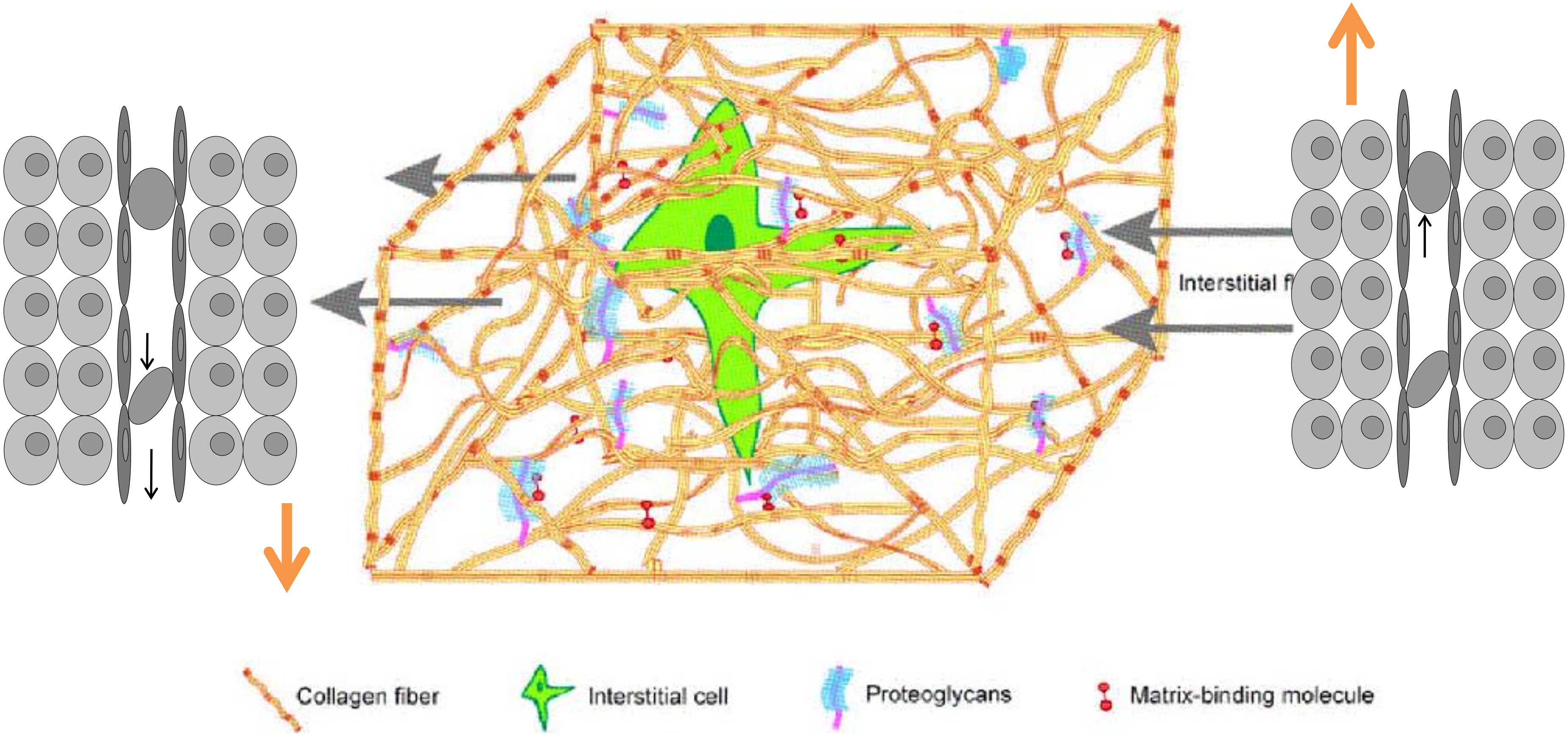
FLOW and SHEAR

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**

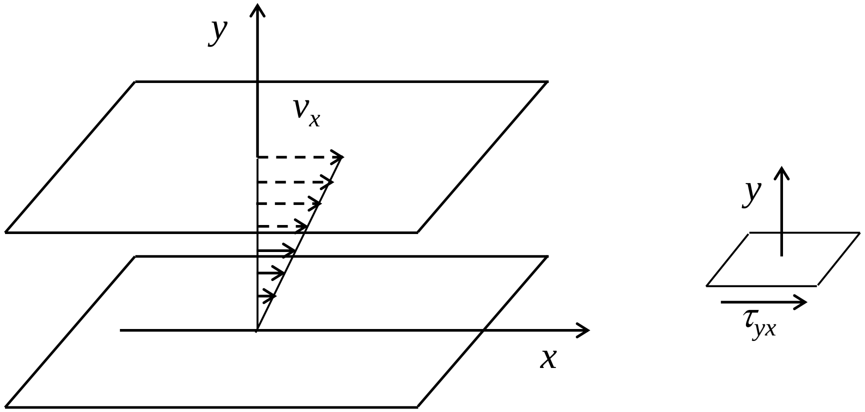
INTERSTITIAL FLOW



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

Shear stress



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

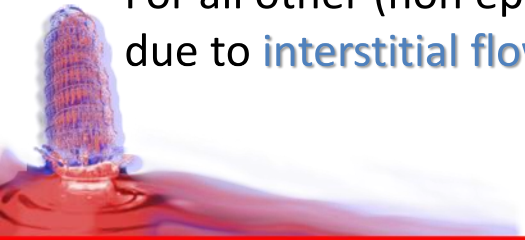
The shear stress on a monolayer of cells in a flat chamber with flow Q is

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

Optimal shear stress in bioreactors

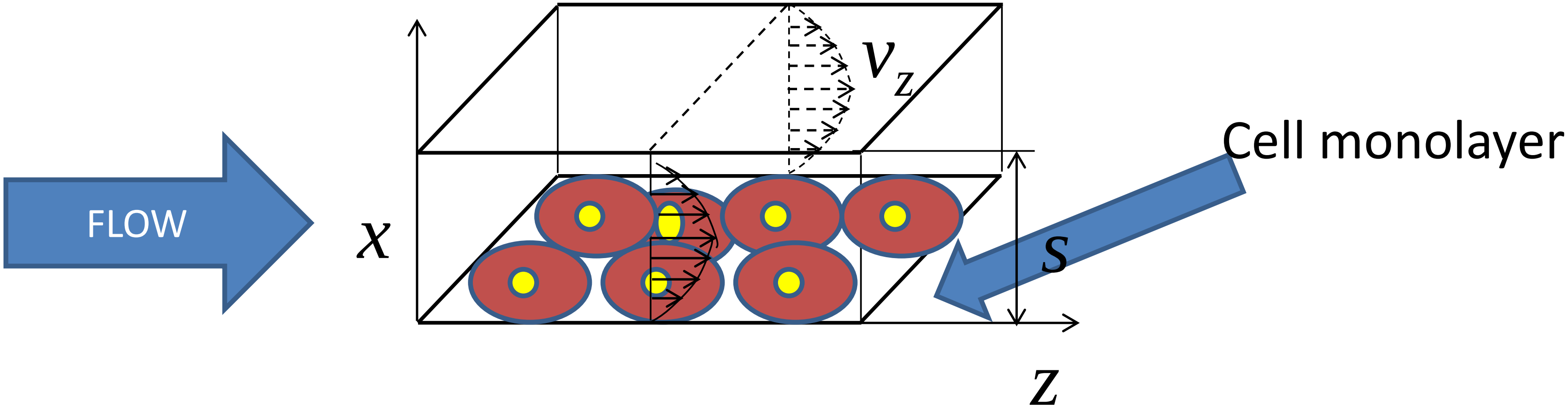
Cell	Shear	Flow rate	Ref
Human trabecular bone, 3D	5.10^{-5} Pa	0.01 mL/min	Porter. Journal of Biomechanics, 38, 543, 2005
Human osteosarcoma cells, 3D	0-0.021 Pa	Max. 25 mL/min	Laganà.Biomedical Microdevices, **14(1), 225, 2012**
hBMSC, 3D	0.015 Pa	3 mL/min	Li. Tissue Eng. A, **15, 2773,2009**
HepG2, 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

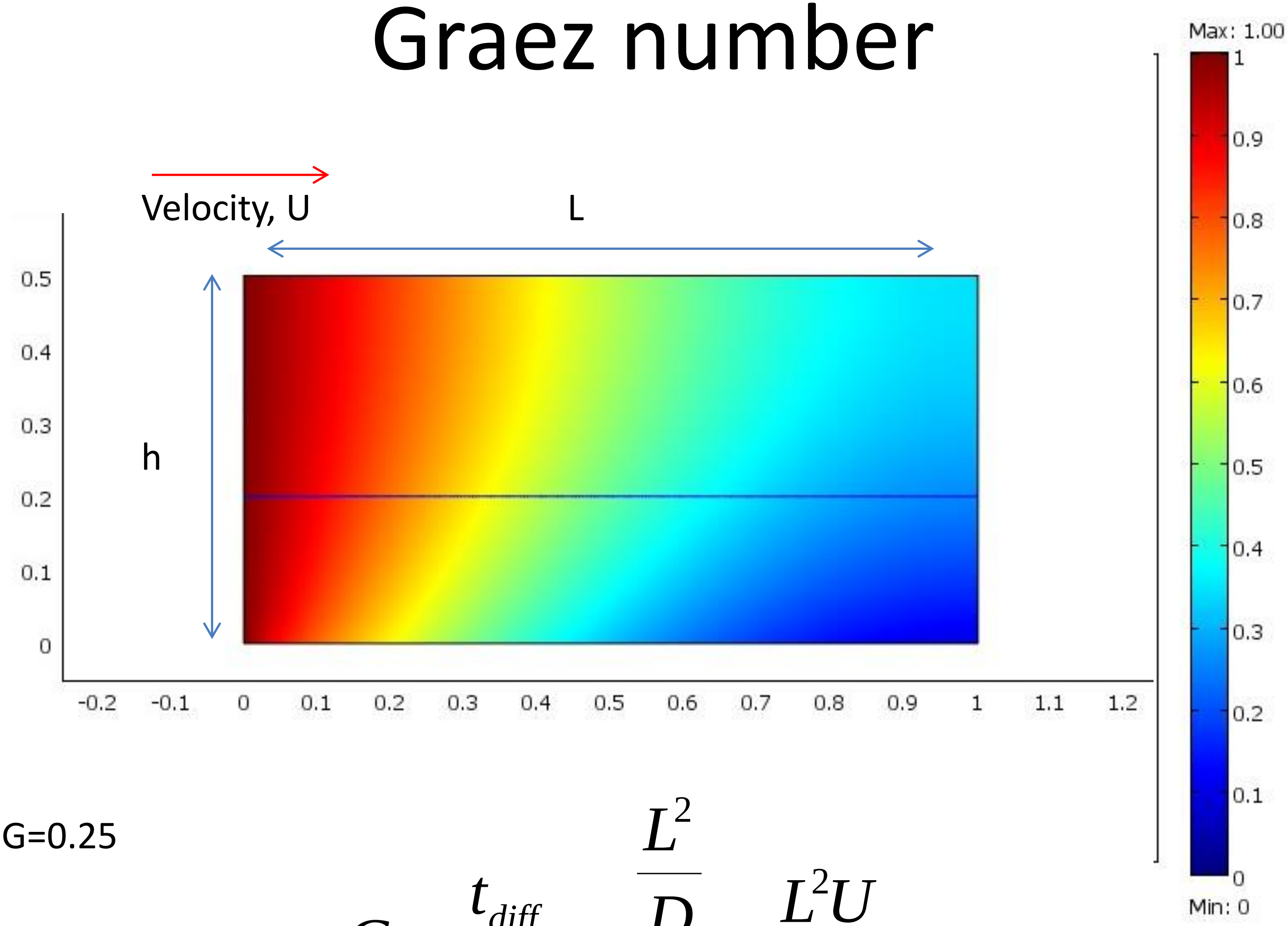
$$\frac{dc}{dt} = D\nabla^2 c - R - v.\nabla c$$



For a monolayer $$\frac{dc}{dt} = D\frac{dc^2}{dx^2} - v_z\frac{dc}{dz}$$

For volumetric consumption $$\frac{dc}{dt} = D\frac{dc^2}{dx^2} - v_z\frac{dc}{dz} - R_{vol}$$

Graez number

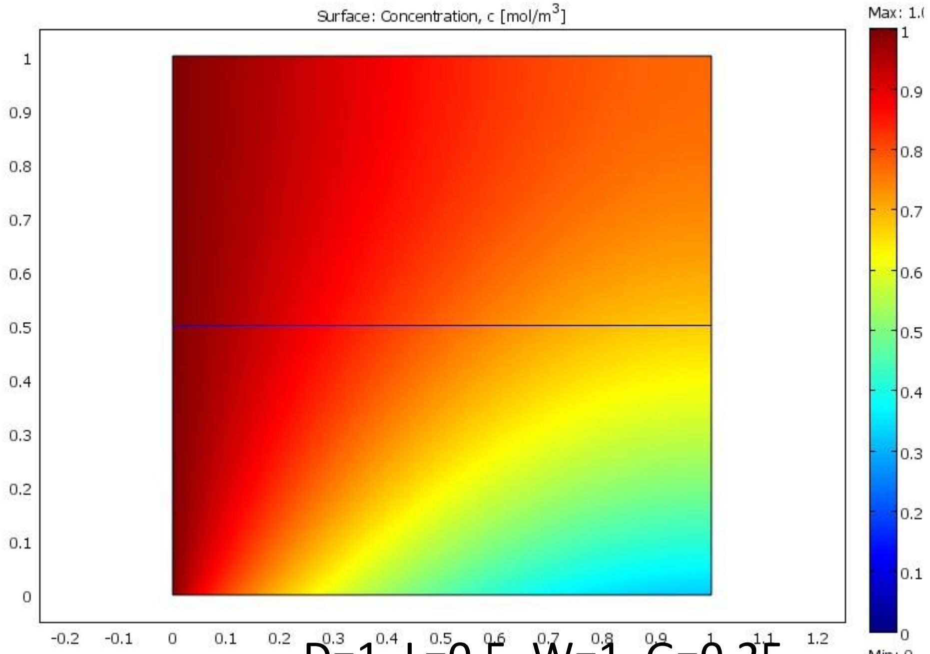


G=0.25

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

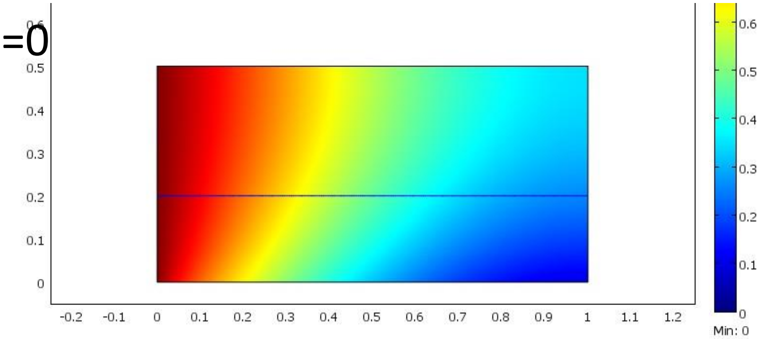
HOW CONCENTRATION PROFILES CHANGE WITH GRAEZ NUMBER

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

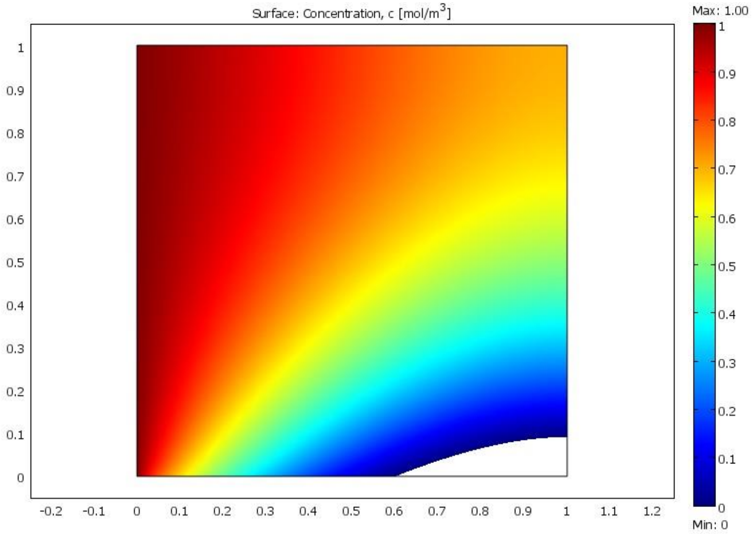


D=1, L=0.5, W=1, G=0.25

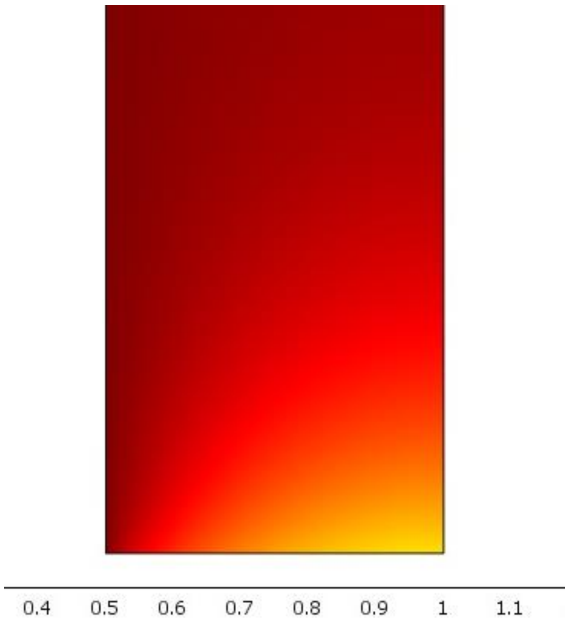
RED, C=1
BLUE C=0

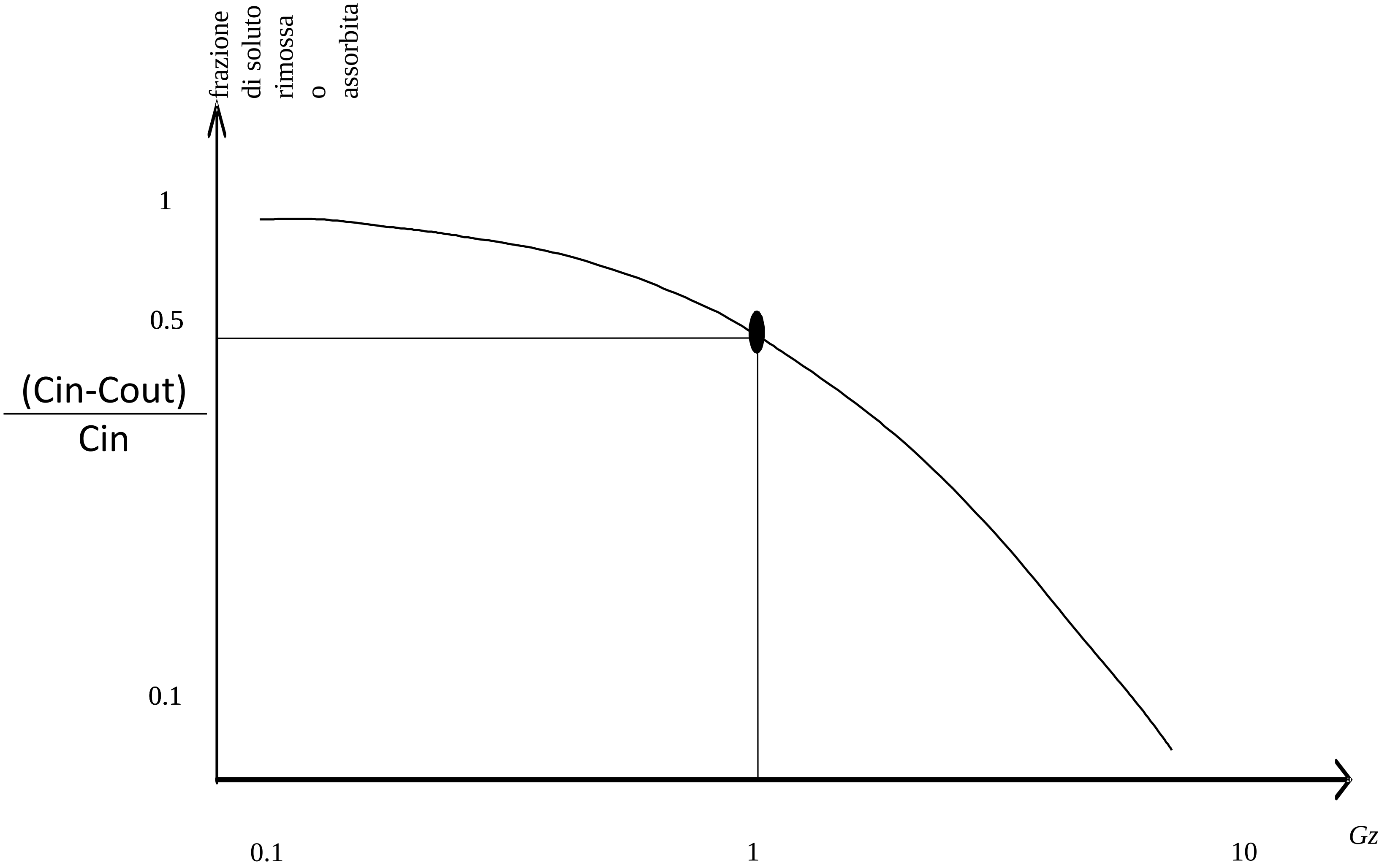


D=2, L=1, W=1, G=0.5



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




frazione
di soluto
rimossa
o
assorbita
1
0.5
(Cin-Cout)
Cin
0.1
0.1
1
10
Gz

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